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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)

RAISED 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_4 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 hear 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_4 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_4 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 bromcresol 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_4 concentration were performed (n = 4). The endogenous FFA concentration in this serum pool was 0.30 mmol/L, and the total T_4 and TBG concentrations were 97 nmol/L and 25.2 mg/L, respectively. The possibility of analytical interference by FFA in the T_4 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_4 6.9, T_4 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_4, 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_4, TBG, and TSH levels and T_4/TBG molar ratios are shown in Table 2. There was a mean 24.8% increase ($P < 0.001$) in serum FT_4 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_4/T_4 mean molar ratios ($\times 10^{-4}$) were 1.23 and 1.26, respectively.

A significant correlation was observed for Δ FT_4 *vs.* Δ FFA ($r = 0.66$, $P < 0.02$) and for Δ FT_4 *vs.* Δ FFA/albumin ratio ($r = 0.75$, $P < 0.01$) (Fig. 1). There was no significant correlation for Δ FT_4 *vs.* Δ TSH ($r = -0.04$, NS) or for Δ T_4 *vs.* Δ FFA ($r = -0.25$; NS).

The following mean serum FT_4 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_4 after the addition of 2.5 mmol/L oleic acid was significant ($P < 0.001$).

Discussion

During ultrafiltration of serum the concentration of T_4-binding proteins in the retentate will not change the ambient FT_4 concentration (31) and centrifugal ultrafiltration has therefore been used by us (6) and others (28–30) for the determination of serum FT_4. 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_4 were due to increased secretion of T_4 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_4. The relative increases in serum total T_4 and TBG were similar and therefore there was no change in the mean T_4/TBG molar ratio indicating that the rise in serum T_4 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_4 to protein binding, pre-

TABLE 1. Serum FT_4, FFA, and albumin concentrations and FFA/albumin molar ratios before (B) and after (A) a prolonged physical exercise in 14 males

Subject no.	FT_4 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_4, TBG, and TSH concentrations and T_4/TBG molar ratios before (B) and after (A) a prolonged physical exercise in 14 males

Subject no.	T_4 B	(nmol/L) A	TBG B	(mg/L) A	T_4/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_4 levels is further evidence against a TSH-induced accelerated thyroidal secretion as the reason for the rise in serum FT_4. Neither did Krotkiewski *et al.* (32) observe an increase in serum T_4 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_4 and FFA indicates that displacement of T_4 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_4 in the subjects studied after prolonged heavy exercise. If the increase in serum FT_4 was caused by a shift of hormone from the intracellular to the extracellular compartment because of displacement of T_4 from cellular stores by FFA, or some other mechanism, there should be an increase in the T_4/TBG ratio. However, the possibility of release of T_4 from intracellular stores cannot be rejected on the basis of the unchanged T_4/TBG molar ratio observed, as competition between FFA and T_4 for binding sites on serum TBG will reduce the T_4/TBG ratio, the net effect possibly resulting in an unaltered ratio. Therefore the possibility of displacement of T_4 from both intra- and extravascular binding sites must be recognized. This could also explain why an expected negative correlation between serum T_4 and FFA could not be detected, although there was a trend. The conclusion that displacement of T_4 from proteins by FFA is a likely mechanism for the 25% rise

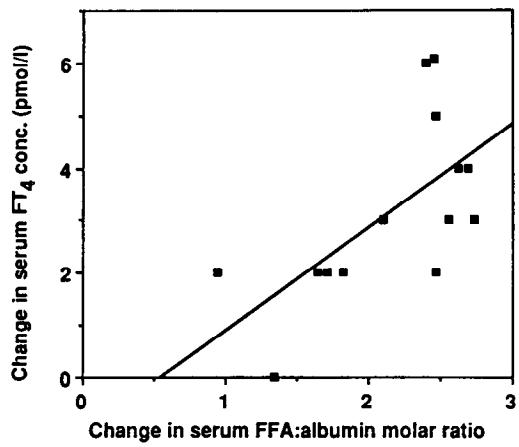

FIG. 1. Relationship between changes in the FT_4 concentration, as determined by direct equilibrium dialysis, and changes in the FFA/albumin molar ratio in sera of males during a prolonged heavy running exercise. Linear regression analysis: $Y = -1.02 + 1.95X$ ($r = 0.75$, $P < 0.01$, $n = 14$).

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.

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