Turnover and Splanchnic Metabolism of Free Fatty Acids in Hyperthyroid Patients

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ABSTRACT The arterial concentration and turnover rate and the splanchnic exchange of FFA were examined after an overnight fast in a group of 11 female patients with clinical and laboratory evidence of hyperthyroidism. [^{14}C]oleic acid was infused intravenously and the hepatic venous catheter technique was used.

As compared with healthy control individuals, the arterial concentrations of FFA and oleic acid were elevated by 30–40% in the hyperthyroid group. Both the turnover rate and the fractional turnover of oleic acid were significantly increased. The turnover rate correlated directly with arterial concentration of oleic acid in both the control and the patient group but the slope was steeper in the patients. The splanchnic uptake of oleic acid was three times higher than in the control group. The augmented uptake was a consequence of elevated arterial concentrations and increased hepatic plasma flow, whereas fractional splanchnic uptake remained unchanged. Ketone body production was four- to fivefold greater in the patients and could be largely accounted for by increased splanchnic FFA uptake. In six patients studied after treatment resulting in a return to normal thyroid function, a significant reduction was observed in arterial FFA, estimated hepatic blood flow, oleic acid turnover, and ketone body production.

It is concluded that hyperthyroidism is characterized by increased turnover and splanchnic uptake of FFA and augmented ketogenesis. These findings can be explained on the basis of elevated arterial FFA concentrations and increased blood flow, particularly to the splanchnic bed.

INTRODUCTION

Hyperthyroidism is accompanied by a marked increase in total energy expenditure as evidenced by clinical symptoms and augmented pulmonary oxygen uptake. Plasma FFA are recognized as the primary fat-derived substrate in man and an important source of energy for the oxidative metabolism of most tissues, particularly liver and muscle. Circulating levels of FFA (1, 2) and glycerol (3) have been shown to be elevated in hyperthyroid patients, indicating an increased rate of FFA release from adipose tissue stores. An increased tendency to develop hyperketonemia during fasting has also been observed in hyperthyroidism (4, 5). However, the effect of spontaneous hyperthyroidism on FFA turnover and the importance of altered arterial FFA levels in determining changes in turnover has not been established. Furthermore, the relative importance of substrate (FFA) delivery vs. altered hepatic metabolism in influencing ketogenesis in hyperthyroidism has not been examined. That is of particular interest because in the diabetic patient, hyperketonemia can not be solely ascribed to increased lipolysis, but is largely dependent on altered hepatic utilization of fatty acids (6).

The present study was consequently undertaken to examine the effects of hyperthyroidism on total and splanchnic turnover of FFA and the relationship of altered substrate delivery to these turnover processes and the production of ketones. In these studies [^{14}C]oleic acid was used as a tracer to determine the turnover of plasma FFA and the hepatic venous catheter technique was used to measure the exchange of FFA and ketone bodies in the splanchnic region. In a previous report data on splanchnic metabolism of glucose and gluconeogenic precursors have been presented (7).

METHODS

Subjects. A group of 11 female patients with clinical and laboratory signs of hyperthyroidism was studied. 10 showed diffuse enlargement of the thyroid and 1 had a multinodular goiter. They all had typical signs of thyrotoxicosis, such as tachycardia, palpitations, heat intolerance, ophthalmopathy, warm and moist skin. Despite an increased appetite, their body weight had fallen 1–5 kg in the 3 mo preceding the

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TABLE I

Age, Body Dimensions, Clinical and Laboratory Findings in Hyperthyroid Patients before and during Treatment

No.	Age	Height	Weight	Duration of symptoms	Triiodothyronine		Thyroxine		BMR	
					Before therapy	During therapy	Before therapy	During therapy	Before therapy	During therapy
	yr	*cm*	*kg*	*mo*	*nmol/liter*		*nmol/liter*		*%*	
1	51	158	43	5	8.4	4.9	296	149	+84	+29
2	40	175	69	5	7.3	2.0	196	119	+64	±0
3	31	167	57	4	6.6	2.5	281	152	+59	+17
4	49	161	55	5	16.4	1.7	373	132	+98	+5
5	35	172	54	6	5.5	2.5	267	133	+79	+18
6	46	158	47	7	5.9	2.1	244	154	+45	−3
7	38	154	61	8	14.8		280		+86	
8	24	164	61	4	8.0		278		+36	
9	47	165	76	10	10.0		224		+32	
10	42	165	50	4	8.4		243		+16	
11	42	160	55	4	11.6		259		+18	

study. Body dimensions and laboratory data are shown in Table I. All subjects were informed of the nature, purpose, and possible risks involved in the study before giving their consent to participate. The protocol was reviewed and approved by the institutional ethical committees. Six patients were restudied during treatment; two of them (patients 1 and 4) received radioiodine (6–9 mCi ^{131}I); the other four received carbimazole (30–40 mg daily). The second study was performed after 5–12 mo of treatment, when the patients were clinically euthyroid. Control data for concentration and turnover of oleic acid in healthy female volunteers (age 24±1 yr, height 166±2 cm, weight 62±2 kg) have been taken from a previous study (8). In the case of splanchnic FFA and ketone body metabolism, the patients have been compared to a group of six healthy male volunteers, age 29±2 yr, height 184±2 cm, and weight 79±5 kg. Previous studies have indicated no differences between males and females with respect to FFA turnover when adjusted for body weight (8).

Procedure. The subjects were studied in the morning after an overnight fast. Catheters were inserted percutaneously into a brachial artery, an antecubital vein; and an hepatic vein. The hepatic venous catheter (Cournand no. 7-8) was placed in a right-sided hepatic vein under fluoroscopic control.

A continuous intravenous infusion of albumin-bound [^{14}C]oleic acid (0.4 μCi/min) was given for 40 min. Three arterial and hepatic venous blood samples were collected at 5-min intervals, starting when the infusion had been running for 30 min. As demonstrated earlier in healthy individuals (9), a constant level of oleic acid specific activity in arterial plasma was reached in each subject during the infusion. This was true in the normal as well as the hyperthyroid subjects in each of whom the three individual values for specific activity fell within 6% of the mean value for the particular subject. Oleic acid-1-^{14}C (sp act 55 mCi/mmol) was prepared and complex-bound to albumin as described previously (10).

Hepatic plasma flow was estimated using continuous infusion of indocyanine green. Plasma volume was calculated as described previously (11).

Analytical methods. Plasma FFA were extracted as described by Dole and Meinertz (12). FFA radioactivity was measured in the heptane extract and subsequently corrected for radioactivity present in esterified fatty acids remaining in the heptane phase after extraction of the free acids into alkaline methanol. The concentrations of individual FFA were determined by gas chromatography, using heptadecanoic acid as internal standard (13, 14). Counting was performed in a Packard TriCarb model 3003 liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.) and the results were corrected for quenching, using internal standards. 3-Hydroxybutyrate and acetoacetate concentrations were determined using enzymatic methods (15). Triiodothyronine (16), thyroxine (17), insulin (18), and glucagon (19) were measured by radioimmunoassay. Glycerol was determined by enzymatic technique (7).

Calculations. The turnover rate of oleic acid was calculated as the rate of infusion of [^{14}C]oleic acid divided by its arterial plasma specific activity. The fractional turnover was obtained as the turnover rate divided by the plasma pool (arterial concentration times plasma volume). The fractional uptake (f) of oleic acid across the splanchnic vascular bed was calculated on the basis of its arterial (A) and hepatic venous (HV) radioactivity: $f = {}^{14}C\text{-}18{:}1_{A-HV}/{}^{14}C\text{-}18{:}1_{A}$. The uptake of oleic acid (U, in micromoles per minute) by the splanchnic area was calculated as the product of f, the arterial plasma concentration of free oleic acid, and the splanchnic plasma flow (P). Release of oleic acid (R, in micromoles per minute) was estimated as the difference between the uptake and the net exchange of unlabeled oleic acid: $R = U - (18{:}1_{A-HV}) \cdot P$.

Standard statistical methods were employed, using the paired *t* test when applicable. Data in the text, tables, and figures are given as mean±SE.

RESULTS

The arterial concentration of total plasma FFA in the patient group (868±68 μmol/liter) was 30% greater than in the controls (669±60 μmol/liter, $P < 0.001$). The increase in concentration of oleic acid was 40% (Table I). The turnover rate of FFA, measured with [^{14}C]oleic acid as tracer, was increased proportionally more than the arterial concentration of FFA, the mean value for the patients being 85% higher than for the controls (Table II). Consequently, the fractional turn-

TABLE II

*Oleic Acid Concentrations and Turnover in Hyperthyroid Patients and Healthy Control Individuals**

	Hyperthyroid patients	Controls‡	Difference
			P
Aterial concentration, *μmol/liter*	373±27	260±24	<0.001
Turnover, *μmol/min*	366±32	197±19	<0.001
Turnover, *μmol/min per kg*	6.3±0.6	3.5±0.3	<0.001
Fractional turnover, *per min*	0.39±0.01	0.33±0.02	<0.001

* Data given as mean±SE.

‡ From reference 8.

over of oleic acid was also significantly augmented in the patient group (Table II). A significant linear relationship was found between oleic acid turnover and its arterial concentration in the patients ($r = 0.94$, $P < 0.001$) as well as in the controls ($r = 0.88$, $P < 0.01$). As a consequence of the higher fractional turnover, the slope of the regression line for the patient group was steeper than for the controls ($P < 0.05$, Fig. 1). Significant correlations were also observed in the patients between oleic acid turnover and heart rate at rest ($r = 0.62$, $P < 0.05$), pulmonary oxygen uptake ($r = 0.78$, $P < 0.01$), and basal metabolic rate ($r = 0.78$, $P < 0.01$, Fig. 2).

Both uptake and release of oleic acid in the splanchnic region were markedly elevated in the patients (Table III). The augmented oleic acid uptake could not

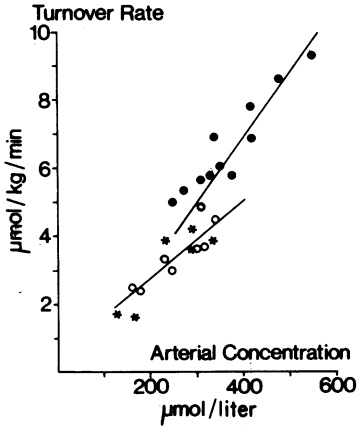

FIGURE 1 Turnover of oleic acid in hyperthyroid patients (●) and controls (○) in relation to the arterial concentration of oleic acid. The solid lines denote the regression lines for the patients ($y = 0.019, x - 0.88, r = 0.94, P < 0.001$) and the controls ($y = 0.011, x + 0.54, r = 0.88, P < 0.01$). Data for six of the hyperthyroid patients after treatment (*) are also shown. These were not included in the regression analysis.

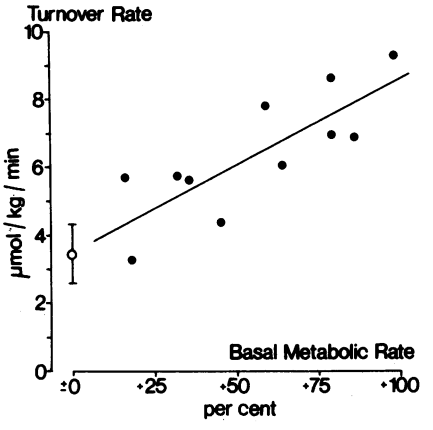

FIGURE 2 Turnover of oleic acid in hyperthyroid patients (●) in relation to their basal metabolic rate ($r = 0.78$, $P < 0.01$). The turnover of oleic acid in the control subjects (○, mean±1 SD) is shown at the theoretical normal mean for basal metabolic rate.

be attributed to an increased extraction of oleic acid, since its fractional extraction was similar in patients and controls. On the other hand, oleic acid delivery to the splanchnic bed was markedly increased.

Arterial concentration of oleic acid was elevated, while hepatic plasma flow was increased in the patients (Table III). Consequently, total substrate (oleic acid) delivery to the splanchnic bed (arterial concentration × plasma flow) in the patient group (7.0±0.9 μmol/min per kg) was substantially greater than that observed in controls (1.5±0.3 μmol/min per kg, $P < 0.001$).

The splanchnic production of ketone bodies was four to five times higher in the patients (Table III). The arterial concentrations of both acetoacetate (0.15±0.03 vs. 0.04±0.01 mmol/liter, $P < 0.01$) and 3-hydroxybutyrate (0.33±0.08 vs. 0.04±0.01 mmol/liter, $P < 0.02$) were also higher than in the controls. The fraction of the splanchnic FFA uptake that was converted to ketone bodies tended to be higher in the patients, but the difference was not significant ($0.05 < P < 0.1$). The fraction of FFA converted to ketone bodies was not significantly related to the thyroid hormone levels ($r = -0.33$, $P > 0.1$) for triiodothyronine and $r = 0.25$, $P > 0.1$ for thyroxine).

As reported previously (7) splanchnic uptake of glycerol was more than fourfold higher in the hyperthyroid group (Table III).

Arterial plasma insulin (16±2 μU/ml) and glucagon (102±13 pg/ml) in the hyperthyroid patients were not significantly different from the corresponding values for the controls (14±3 μU/ml), and 62±14 pg/ml, respectively).

Six hyperthyroid patients were reexamined when treatment had resulted in a return of thyroid hormone

TABLE III

*Splanchnic Metabolism of Free Oleic Acid, Ketone Bodies, and Glycerol in Hyperthyroid Patients and Healthy Control Individuals**

	Hyperthyroid patients	Controls	Difference
			P
Oleic acid uptake, *μmol/min per kg*‡	2.04±0.18	0.50±0.08	<0.001
Oleic acid release, *μmol/min per kg*	0.47±0.07	0.12±0.03	<0.005
Estimated hepatic plasma flow, *ml/min per kg*	18±1	12±1	<0.005
Fractional uptake of oleic acid	0.31±0.02	0.36±0.03	NS
3-hydroxybutyrate output, *μmol/min per kg*	4.8±1.2	0.5±0.1	<0.02
Acetoacetate output, *μmol/min per kg*	3.4±0.8	0.6±0.1	<0.02
Ketone body output/FFA uptake§	0.37±0.07	0.20±0.05	NS
Glycerol uptake, *μmol/min per kg*	2.4±0.3	0.5±0.03	<0.001

* Values are given as mean±SE.

‡ Oleic acid uptake was calculated as the product of the fractional uptake of oleic acid, the arterial concentration of oleic acid and splanchnic plasma flow. The arterial oleic acid concentration in the hyperthyroid group is shown in Table II; for the control subjects studied by hepatic vein catheterization, arterial oleate was 129±25 μmol ($P < 0.001$ vs. hyperthyroid group).

§ Splanchnic FFA uptake was calculated from oleic acid uptake and the percentage oleic acid in plasma FFA. For comparison with the ketone body output it was converted into four-carbon equivalents, assuming a mean chain length of 17.4 carbons per fatty acid.

levels and basal metabolic rate to normal in all patients except one (Table I). In the latter patient (No. 1) a considerable reduction in these parameters had been achieved, but she still had a slightly elevated serum triidothyronine level and basal metabolic rate. The reexamination showed that for the whole group arterial plasma FFA had decreased from 948±104 to 588±72 μmol/liter ($P < 0.05$). Both turnover rate and fractional turnover of oleic acid were also reduced after treatment (Table IV) and the relation between turnover rate and arterial concentration was now similar to that in the controls (Fig. 1). Estimated hepatic blood flow was significantly lower after treatment and the splanchnic uptake and release of oleic acid as well as the splanchnic production of ketone bodies had decreased (Table IV). The average fraction of splanchnic FFA uptake converted to ketone bodies tended to decline after treatment, but this difference was not significant ($0.05 < P < 0.1$).

DISCUSSION

The present findings demonstrate that the arterial concentration as well as the turnover rate of oleic

TABLE IV

*Effect of Treatment on Oleic Acid and Ketone Body Metabolism in Six Hyperthyroid Patients**

	Before treatment	After treatment	Difference
			P
Arterial concentration oleic acid, *μmol/liter*	403±42	239±32	<0.05
Turnover rate oleic acid, *μmol/min*	393±44	194±35	<0.01
Turnover rate oleic acid, *μmol/min per kg*	7.1±0.8	3.1±0.5	<0.01
Fractional turnover oleic acid, *min*	0.40±0.01	0.30±0.02	<0.02
Estimated hepatic plasma flow, *ml/min per kg*	19±2	14±1	<0.05
Splanchnic oleic acid uptake, *μmol/min per kg*	2.2±0.2	1.0±0.2	<0.01
Splanchnic oleic acid release, *μmol/min per kg*	0.7±0.1	0.3±0.1	<0.05
Splanchnic 3-hydroxybutyrate output, *μmol/min per kg*	6.8±1.8	1.9±0.8	<0.05
Splanchnic acetoacetate output, *μmol/min per kg*	4.7±1.1	1.8±0.5	<0.05
Ketone body output/FFA uptake‡	0.50±0.09	0.32±0.08	NS
Glycerol uptake, *μmol/min per kg*	2.8±0.5	1.1±0.05	<0.02

* Values are given as mean±SE.

‡ Splanchnic FFA uptake was calculated from oleic acid uptake and the percentage oleic acid in plasma FFA. For comparison with the ketone body output it was converted into four-carbon equivalents, assuming a mean chain length of 17.4 carbons per fatty acid.

acid are significantly elevated in patients with clinical and laboratory evidence of hyperthyroidism. The higher oleic acid levels confirm previous observations of increased FFA concentrations in hyperthyroidism (1, 2). The current results are also in accord with the observation that FFA turnover is elevated in experimental thyrotoxicosis induced in man by large doses (300–500 μg/d) of triiodothyronine (20). The present findings extend those observations by (*a*) indicating that turnover of FFA is increased in spontaneous hyperthyroidism, (*b*) examining the factors that may be responsible for the increase in turnover and (*c*) examining the effects of hyperthyroidism on splanchnic metabolism of FFA and ketones.

In the hyperthyroid group the increase in turnover of oleic acid could be attributed to an augmented rate of release of FFA from adipose tissue as reflected by the higher arterial concentrations, as well as an increase in utilization of available FFA as indicated by the higher fractional turnover rate of oleic acid in the patient group (Table II). This was also reflected by the steeper slope of the regression line relating turnover rate to arterial concentration in the patient group as compared with the control individuals (Fig. 1).

With respect to the mechanism of the increase in fractional turnover of oleic acid, circulatory factors may be of major importance. Thus, muscle uptake of FFA at rest and during exercise has been demonstrated to depend not only on arterial concentration, but also on blood flow to the exercising muscles (10, 21, 22). Furthermore, the increase in the fractional turnover of FFA during exercise is related to the increased cardiac output (21, 23). A hyperkinetic circulation with an increased heart rate and cardiac output are typical findings in hyperthyroidism (24). Although cardiac output was not measured in the present study, it is likely that the patients showed moderate to marked degrees of hyperkinetic circulation; their average heart rate at rest was 90±5 beats/min. In addition, a significant relationship was observed between oleic acid turnover and heart rate, thus providing support for the notion that circulatory factors contribute to the augmented FFA utilization in hyperthyroid patients. On the other hand, the increase in FFA utilization may not be causally related to changes in the circulation but both may be secondary to increased energy demands in hyperthyroidism.

As to the mechanism of increased FFA mobilization in the hyperthyroid group, circulatory as well as hormonal factors may be contributing. Hyperthyroid patients have an augmented subcutaneous blood flow (25) to meet the demand for increased heat dissipation and this may increase the rate of FFA mobilization from adipose tissue. In contrast, thyroid hormones have not been shown to have any direct lipolytic effect and the basal rate of lipolysis is unaltered when adipose tissue obtained from hyperthyroid patients is incubated in vitro (26). On the other hand, thyroid hormone does increase the lipolytic response of adipose tissue in vitro to catecholamine stimulation (26, 27, 28). While insulin is recognized to be a potent antilipolytic hormone, it is noteworthy that augmented lipolysis occurred in the thyrotoxic group in spite of insulin levels that were no different from the controls.

The overall increase in oleic acid turnover observed in the thyrotoxic group was directly related to the rise in metabolic rate (Fig. 2). However, when total FFA turnover is estimated from the oleic acid data and converted to oxygen equivalents, it is calculated to represent 161±10% of the total pulmonary oxygen uptake. This value is markedly higher than that observed in controls (82±19%, $P < 0.01$). The fact that FFA turnover is substantially in excess of that attributable to oxidation is compatible with earlier studies indicating that synthesis of triglyceride from free fatty acids is augmented in the thyrotoxic state (29). Thus, the overall acceleration in FFA turnover in thyrotoxicosis represents not only augmented utilization for oxidative purposes but also increased flux into triglycerides. In keeping with the latter conclusion was the finding that splanchnic glycerol uptake was increased fourfold in the hyperthyroid group.

Splanchnic uptake of oleic acid was three times higher in the hyperthyroid patients and was attributable to the elevated arterial concentration and increased hepatic blood flow. In contrast, splanchnic fractional extraction of oleic acid was comparable to controls (Table III). In a similar manner the fall in splanchnic uptake of oleic acid observed after treatment was entirely attributable to the fall in arterial levels and hepatic blood flow (Table IV). Thus tissue presentation of FFA (arterial concentration × plasma flow) primarily determines the rate of splanchnic uptake. In this respect hyperthyroid patients did not differ from the controls.

Ketone body production was markedly elevated in the patient group (Table III). This could mainly be explained by the increased uptake of FFA. The data, however, also suggest the possibility of an intrahepatic effect since the mean fraction of FFA uptake that was converted to ketone bodies was 0.37 in the patients and 0.20 in the controls. (Table III) and fell from 0.50 to 0.32 in the patients studied before and after treatment (Table IV). Although these changes were not statistically significant, they do raise the possibility that in hyperthyroidism intrahepatic disposal of FFA may be altered so as to favor ketone body production.

With regard to hepatic ketogenesis it is interesting to compare the present results with those we obtained in diabetic subjects deprived of insulin for 24 h (9). The diabetics had arterial FFA levels (791±89 μmol/

liter) comparable to those observed in the present study. Splanchnic uptake of oleic acid was, however, 76±11 μmol/min, i.e., only 65% of the value in the hyperthyroid patients. Despite this, the splanchnic production of ketone bodies in the diabetics was 30% greater than in the hyperthyroid patients. The fraction of splanchnic FFA uptake that was converted into ketone bodies was 0.66 in the diabetics compared with the present observation of 0.37. Furthermore, in the diabetics who had developed ketosis (total ketone bodies > 2 mmol/liter) this fraction was even higher (0.85). These findings are in accord with the concept that development of ketosis in the diabetic requires not only augmented adipose tissue lipolysis but also intrahepatic activation of fatty acid oxidation (6). The latter process appears to be only partially activated in hyperthyroidism. The lower rate of ketone body production in the hyperthyroid group as compared to diabetics may also be a consequence of an increased rate of FFA re-esterification to triglycerides that, as noted above, is suggested by the relation between splanchnic oxygen consumption and FFA uptake as well as the increase in glycerol uptake.

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