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CLINICAL STUDY

Changes in renal tri-iodothyronine and thyroxine handling during fasting

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Abstract

Objective: Liver handling of thyroid hormones (TH) has been known to alter significantly during fasting. This study investigates whether renal handling of TH is also changed during fasting.
Methods: We measured urinary excretion rates and clearances of free tri-iodothyronine (T_3) and free thyroxine (T_4) in healthy subjects prior to and on the third day of fasting.
Results: During fasting, both mean T_3 and T_4 urinary excretion decreased significantly to a mean value of 42% of control. Also, total and free (F) serum T_3 concentrations declined significantly, but serum T_4 did not change. Both FT_3 and FT_4 clearance decreased significantly during fasting (62% and 42% of control). The fasting-induced decrease in uric acid clearance correlated well with the decrease in FT_3 clearance ($r = 0.94$; $P < 0.001$). Serum concentrations of non-esterified fatty acids (NEFA) were significantly elevated during fasting.
Conclusions: The findings cannot be fully explained by the fasting-induced decrease in serum T_3, and are in accordance with inhibition of uptake of T_3 and T_4 at the basolateral membrane of the tubular cell. This inhibition may be caused by a decreased energy state of the tubular cell and by other factors such as ketoacidosis and/or increased NEFA concentrations during fasting.

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Introduction

In man, eighty percent of plasma tri-iodothyronine (T_3), the bioactive thyroid hormone, is produced outside the thyroid by deiodination of thyroxine (T_4), which is the main secretory product of the thyroid gland (1). Both liver and kidney cells contain high amounts of the enzyme, type I 5′-deiodinase (2, 3), that converts T_4 to T_3. However, before this intracellular conversion can take place, entry over the plasma membrane into liver and kidney cells has to occur. We showed that uptake of T_4, but also of T_3 and reverse (r) T_3 into liver cells is mediated by an active transport process that is dependent on ATP and on the Na^+-gradient (4). This process appeared to be the rate-limiting step in the metabolism of thyroid hormones (5).

During fasting and non-thyroidal illness (NTI), a decrease in serum T_3 concentration, a rise in rT_3 and mostly unchanged free (F) and total (T) serum T_4 concentrations are seen (6). These alterations in serum thyroid hormone levels are explained by unchanged plasma T_4 and rT_3 production rates, combined with both decreased T_4 to T_3 conversion and rT_3 degradation in the liver (6). In addition to decreased 5′-deiodinase activity, catalyzing both T_4 to T_3 formation and rT_3 degradation (7), decreased uptakes of T_4 and rT_3 into the liver have been described as causes for these alterations (6). In a study using a three compartment model for thyroid hormone kinetics, we showed that during caloric deprivation, transport of T_4 into the fast equilibrating compartment (composed of liver and kidney) was diminished (8), resulting in a decline of substrate for T_3 production and consequently leading to a lowered plasma T_3 production rate.

Much attention has been given to the changes in liver handling of thyroid hormones seen in NTI and during fasting (6, 9, 10). However, it is not known whether changes in renal handling of thyroid hormones occur during fasting and NTI. In this study, we aim to illuminate some aspects of renal excretion of thyroid hormones during fasting in healthy subjects, thus creating an experimental form of NTI.

Materials, subjects and methods

Subjects

Four (2 female) healthy volunteers aged 20 to 25 years participated in this study. Thyroid function as evaluated by serum concentrations of TT_4, FT_4, TT_3, FT_3, rT_3 and thyrotropin (TSH) was normal in all subjects. Apart from oral contraceptives used by subject 4, no medication was taken. Indices of nutritional status prior to the fast were normal in all subjects studied (normal serum concentrations of T_3, creatinine, uric acid, urea (5.65 ± 1.06 mmol/l) and free fatty acids).

Study design

On day 0 a normal diet was consumed. Urine was collected for 24 h and a blood sample was taken. From day 1 to 3, the subjects fasted, but a maximal caloric intake of 60 kcal per day in the form of low fat bouillon was allowed. On day 3, another 24-h urine sample was collected and a blood sample was taken. All blood samples were taken at the mid-point of a 24-h collection period.

Urine analysis

Samples of the 24-h urine collection were stored at -20 °C until analysis. Uric acid, creatinine and urea concentrations were measured on an EPOS analyzer from Eppendorf (Hamburg, Germany). Urinary T_4 and T_3 concentrations were determined by RIA. Using a highly specific antibody, no thyroid hormone conjugates could be measured. In a set of 3 experiments, the recovery of thyroid hormone added to charcoal-treated urine was measured. A regression line: $y = 0.97x$-0.06, $P = 0.04$ was obtained. Changing the urinary pH by adding 50 μl of either 0.1 mol/l NaOH or 0.1 mol/l HCl to 950 μl urine did not influence the recovery of added thyroid hormone.

Serum analysis

Serum was stored at -20 °C until analysis. Serum TT_4, TT_3, and rT_3 were determined by RIA. Serum FT_4 was determined using an Amerlite kit from Johnson and Johnson Clinical Diagnostics Ltd (Amersham, Bucks, UK). The serum FT_3 fraction was determined by equilibrium dialysis according to Sterling and Brenner (11), with minor modifications (12). Serum TSH was determined using a TSH Amerlite kit (Johnson and Johnson Clinical Diagnostics Ltd). Urea, creatinine and uric acid were determined on a Chem 1 analyzer from Technicon (Tarrytown, NY, USA). Serum non-esterified fatty acids (NEFA) were measured using a NEFA C-test (Wako Chemicals, Tokyo, Japan).

Calculations and statistical analysis

Clearances of creatinine, uric acid, FT_3 and FT_4 were calculated using the formula U*V/P*1.44, where U is urinary concentration in mol/l, V is urinary volume of 24-h collection period in ml/24 h and P is plasma concentration in mol/l (13).

The significance of differences was determined by Student's *t*-test for paired observations. All data are expressed as means ± S.D. A P value < 0.05 was considered statistically significant.

Results

Table 1 shows the parameters measured to evaluate thyroid function before and on the third day of fasting. Data on all subjects follow the same trend. T_3 concentrations are significantly lowered, and those of rT_3 are elevated after fasting. Total and free T_4 concentrations are unchanged. Serum TSH levels decline but all values remain within the reference range.

In Table 2, urinary excretion of T_3 and T_4 on both the control day and the third day of fasting are shown. Urinary excretion of both T_3 and T_4 is significantly diminished during fasting. Since urinary excretion is dependent on the filtration of plasma free hormone, changes in plasma concentrations will lead to changes in urinary excretion. Therefore, renal clearances of FT_3 and FT_4 are given. In the control period, renal clearance of FT_3 is significantly greater and that of FT_4 significantly smaller than the creatinine clearance. Fasting causes a decline in both renal clearances,

Table 1 Serum thyroid function parameters prior to and on the third day of fasting. Data represent the mean ± S.D. of 4 healthy subjects, except for serum rT_3 data: $n = 3$, before and on the third day of fasting.

	Control	Fasting	Mean % of control
TSH (mU/l)	0.88 ± 0.46	0.37 ± 0.23[a]	42.3
TT_4 (nmol/l)	103.3 ± 24.8	102.8 ± 26.6	99.4
FT_4 (pmol/l)	19.4 ± 5.4	18.9 ± 3.9	98.5
T_3 (nmol/l)	1.93 ± 0.53	1.29 ± 0.32[b]	67.8
FT_3 (pmol/l)	4.2 ± 0.6	2.9 ± 0.7[c]	68.2
rT_3 (nmol/l)	0.27 ± 0.06	0.53 ± 0.16[d]	194.8

[a] $P = 0.0734$; [b] $P = 0.0291$; [c] $P = 0.0184$; [d] $P = 0.05$ versus control period data.

Table 2 Urinary excretion values (in pmol/24 h) of T_4 and T_3 and the clearances (in ml/min) of FT_3, FT_4 and creatinine prior to and on the third day of fasting. Data represent the mean ± S.D. of 4 healthy subjects before and on the third day of fasting.

	Control	Fasting	Mean % of control
T_3 excretion	1367 ± 273	559 ± 96[a]	41.7
FT_3 clearance	224.5 ± 24.6[d]	138.8 ± 31.2[a,f]	61.7
T_4 excretion	1826 ± 702	783 ± 535[b]	41.6
FT_4 clearance	70.3 ± 35.7[e]	30.8 ± 22.8[c]	41.9
Creatinine clearance	121 ± 6.6	111.7 ± 23.0	93.6

[a] $P = 0.007$; [b] $P = 0.0123$; [c] $P = 0.0231$ versus control period data. [d] $P = 0.0048$; [e] $P = 0.02$; [f] $P = 0.1616$ versus creatinine clearance in the same period.

resulting in a FT_3 renal clearance that is no longer statistically different from the creatinine clearance ($P = 0.1616$).

In Table 3, serum concentrations, 24-h urinary excretion rates and clearance of uric acid are given. Serum uric acid concentrations rise significantly during fasting, but urinary excretion and clearance of uric acid decrease significantly. Figure 1 shows the correlation between renal clearances of FT_3 and uric acid ($r = 0.94$, $P < 0.001$).

Serum NEFA levels, measured in 3 subjects, rise significantly after 3 days of fasting (0.27 ± 0.22 mmol/l in the control period versus 1.03 ± 0.46 mmol/l in the fasted state, $P = 0.0305$).

Discussion

In this study, we investigated renal handling of thyroid hormones during fasting in man. Our control values of T_4 and T_3 excretion and renal clearances of these hormones in healthy euthyroid persons are in good agreement with reported values obtained in other studies (14–20). The FT_3 renal clearance in healthy humans is significantly greater than the creatinine clearance, the latter being a measure of the glomerular filtration rate (GFR), whereas FT_4 clearance is significantly lower, as has been found by others (14–20) and in this study. These data are consistent with active (tubular) excretion of T_3 into the urine and effective reabsorption of T_4 from the glomerular filtrate in the fed man.

We showed a dramatic decline in urinary excretion of both T_3 and T_4 during fasting. Since the urinary fate of a substance is dependent on its serum concentration, calculation of the renal clearance is needed to correct for differences in serum concentrations. When calculating the renal clearances, we and others (17–20) used the serum free hormone concentrations since only the free fraction of these hormones is available for glomerular filtration and cellular uptake. The decrease in FT_3 and FT_4 clearance during fasting indicates that, in addition to decreased T_3 serum concentrations, other factors are responsible for the lowered excretion of thyroid hormones into the urine.

Figure 2 depicts a schematic proximal tubule cell of the kidney. Urinary T_3 and T_4 may be considered as the sum of glomerularly filtered thyroid hormone (TH) (pathway 1), plus TH excreted to the urine (pathway 2), minus reabsorbed TH (pathway 3). Glomerular filtration of free TH results in a free hormone concentration, at the start of the proximal tubule, equal to that in serum. Reabsorption of both T_4 and T_3 occurs subsequently, but also uptake at the basolateral membrane of the proximal tubule cell takes place. Cavalieri *et al.* (21) showed that, in humans, the unidirectional renal T_3 uptake rate is about 50 nmol per day. This rate is far greater than the filtered load per day, which is around 0.8 nmol per day (serum FT_3 times GFR, this study). Furthermore, Ferguson *et al.* reported unchanged T_4 uptake in the isolated perfused rat kidney after a decrease in the GFR (22). These observations indicate that thyroid hormones may be taken up at the basolateral membrane of the tubule cell and/or in other kidney cells. Thus, in the fed condition, uptake of TH takes place at both the luminal membrane (reabsorption, pathway 3) and the basolateral membrane (pathway 4). Deiodination of T_4 to

Table 3 Serum concentrations, 24-h urinary excretion rates and clearance of uric acid (UA) prior to and on the third day of fasting. Data represent the mean ± S.D. of 4 healthy subjects before and on the third day of fasting.

	Control	Fasting	Mean % of control
Serum UA (mmol/l)	0.28 ± 0.06	0.46 ± 0.07[a]	168.5
UA excretion (pmol/24 h)	3.37 ± 0.82	1.27 ± 0.75[b]	40.6
UA clearance (ml/min)	8.65 ± 1.74	2.05 ± 1.41[c]	23.4

[a] $P = 0.0002$; [b] $P = 0.009$; [c] $P = 0.0049$ versus control period data.

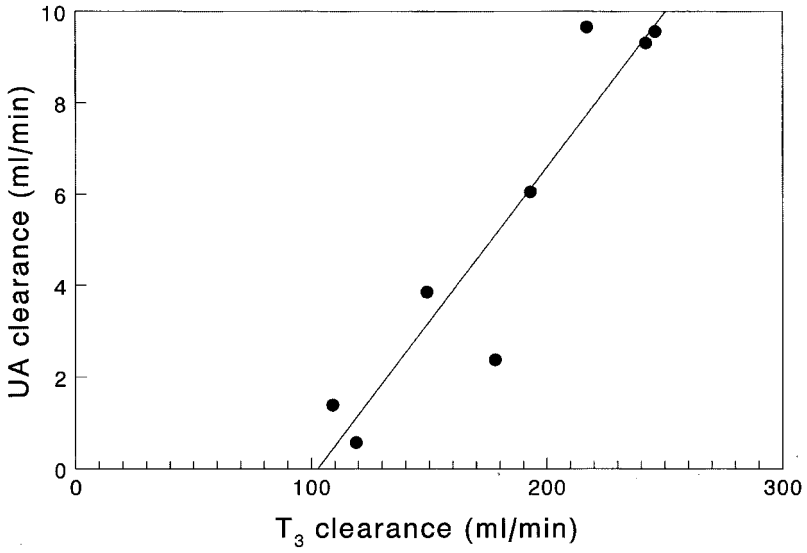

Figure 1 Plot of the correlation between uric acid (UA) clearance and FT_3 clearance measured in 4 healthy subjects prior to and on the third day of fasting. $r = 0.94$, $P < 0.001$.

T_3 (pathway 5), excretion into the urine (pathway 2) or secretion to the blood (pathway 6) may follow subsequently.

The lowered excretion and clearances of T_3 and T_4 during fasting may either be caused by stimulation of pathway 3, or by a decrease in pathway 2. It is unlikely that in a state of energy depletion, such as fasting, pathway 3 will be stimulated, as during caloric deprivation in humans uptake in the rapid equilibrating compartment (liver and kidney) is decreased (8). Therefore, a decrease in pathway 2 may be a much more likely explanation for the findings in this study. In a recent study, we showed that cellular efflux of thyroid hormones is a passive process, not influenced by changes in cellular ATP content, and driven by the intracellular free concentration of the hormone (23). Thus, a lowered rate of efflux via pathway 2 is consistent with a lowered intracellular free hormone concentration. This may be explained by a lowered basolateral uptake (pathway 4) and/or by a lowered luminal uptake (pathway 3).

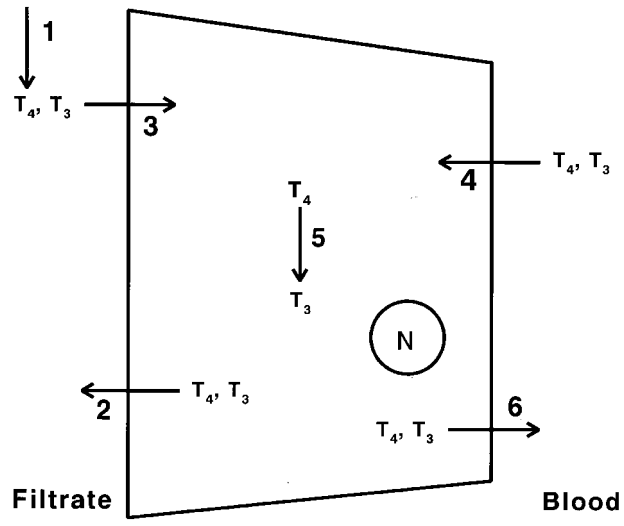

Figure 2 Schematic representation of a proximal tubule cell of the kidney. N = nucleus. For explanation of the different pathways (1–6), see Discussion.

Inhibition of pathway 3 would result in augmented excretion of TH into the urine, which is evidently not the case in our study. Thus, it remains that inhibition of pathway 4 may be the cause for the decrease in pathway 2 via a decrease in intracellular free hormone concentration.

We showed that uptake of T_3 is diminished in perfused livers of fasted rats. Re-perfusion with energy-enriched perfusion medium restored T_3 uptake within half an hour (7), suggesting involvement of ATP in the transport process that is being lowered during fasting. In liver perfusions with fructose in the medium, ATP concentrations were diminished drastically and T_3 uptake decreased in parallel (10). These studies indicate that cellular ATP is a major determinant of cellular thyroid hormone uptake, as was found earlier in rat and human hepatocytes in culture (24, 25). In the present study, cellular kidney tubular ATP may also be lowered due to fasting.

As a result of fasting, serum concentrations of NEFA rise, as reported earlier (26, 27). In a study with obese subjects undergoing caloric restriction, elevated NEFA concentrations in the sera of these subjects were found to be responsible for the inhibited T_4 uptake into rat hepatocytes (27). Thus, elevated NEFA concentrations may, in addition to lowered cellular energy, be responsible for the suggested inhibition of pathway 4 (Fig. 2).

In the proximal tubule, uric acid is both actively reabsorbed and secreted into the urine (28, 29). In the fasted state, a developed ketoacidemia alters renal

clearance of uric acid by an effect on these tubular transport mechanisms (28, 29). These alterations cause a lowered urinary excretion, with subsequent elevated serum concentrations of uric acid (26, 28, 29). The correlation between FT_3 renal clearance and uric acid clearance suggests that tubular handling of T_3 and uric acid may both be affected by similar mechanisms such as ketoacidosis or a decrease in cellular energy stores having similar or different transport pathways.

In this study, we used creatinine clearance (CrCl) as a marker for GFR, since CrCl values have been shown to correspond well with measured inulin clearances in fasting subjects on the third day of fasting in studies by others (30–32). In the studies of Fox *et al.* (28) and Hoffman *et al.* (33), GFR remains stable over three days of fasting, as in our study.

One of our subjects used oral contraceptives. These drugs are known to alter protein binding of thyroid hormones. Therefore, TT_3 and TT_4 were elevated, whereas free concentrations were normal. It can be questioned whether this medication may have induced alterations in renal handling of T_3 and T_4 in one of our fasting subjects. However, Gaitan *et al.* (15) reported unchanged urinary T_3 excretion in subjects using oral contraceptives, so it is unlikely that this drug interfered in our study, especially since excretion and clearance values of this subject were comparable to those of the other subjects.

In conclusion, fasting induced a significant decline in both T_3 and T_4 urinary excretion and renal clearances, which is, in addition to a decrease in serum FT_3, consistent with inhibited uptake of T_3 and T_4 at the basolateral membrane of the proximal tubular cell. This uptake inhibition may be explained by lowered energy stores of the tubular cells or by other factors such as ketoacidosis and the detected elevation of serum NEFA levels.

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