Problems in Estimating Thyroxine Secretion Rates in Cattle

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Abstract

Experiments with dairy cows evaluated thyroxine secretion rates calculated on plasma disappearance rates of iodine from radioactive thyroxine, chemical thyroxine, and protein-bound iodine. Variations in estimated thyroxine secretion rates occurred in the same cows due to different rates of disappearance and, apparently, different volumes of distribution of test doses between different organic iodine sources. After a large intravenous dose of thyroxine, iodine from radioactive thyroxine and chemical thyroxine disappeared from plasma faster than protein-bound iodine. High iodine diets reduced disappearance rates of protein-bound iodine more than that of thyroxine. Feeding 100 milligrams iodine daily caused normal thyroxine determined in plasma protein-bound iodine to decline from 88 to 59%. Injecting 20 milligrams of 1-thyroxine intravenously caused thyroxine iodine to exceed protein-bound iodine for 1 day on high iodine diets and 2 days on low iodine diets. Protein-bound iodine cannot be used as a precise index of plasma thyroxine. Most accurate estimations of thyroxine secretion rates are made by injecting a minimum dose of radioactive thyroxine to determine rate of disappearance and thyroxine space and determine plasma thyroxine by a method specifically for chemical thyroxine.

Introduction

Thyroxine, the major hormone secreted by thyroid glands, exerts a profound effect upon energy metabolism and, consequently, animal productive processes. A certain level of thyroxine, the euthyroid condition, is also necessary for normal functioning of many other hormone and metabolic systems. Production and release of thyroxine by the thyroid gland is controlled by the anterior pituitary through its thyroid stimulating hormone regulated primarily by concentration of thyroxine or triiodothyronine in the plasma. Each animal has its own internal regulating system to maintain a constant rate of release of thyroxine dependent upon utilization rate. Estimation of thyroxine secretion rate (TSR) in different types of animals under varying environmental and physiological conditions has been the objective of numerous investigations (1, 2, 4, 5, 6, 9, 10, 13).

Three different methods of estimating TSR have been proposed: substitution or injection of exogenous thyroxine in sufficient minimal daily doses to stop thyroxine release from the thyroid, determination of thyroxine utilization rate after intravenous injection of exogenous thyroxine by measuring pool size and rate of decline of either plasma protein-bound iodine (PBI) or thyroxine, and determination of thyroxine utilization rate from disappearance rate and pool size of a tracer dose of radioactive thyroxine. Post and Mixner (9) showed the three methods gave essentially the same TSR for dairy cows or bull calves. They compared the same animals in which chemical thyroxine turnover tests immediately followed radioactive thyroxine turnover tests and found that a single dose of 25 mg of 1-thyroxine failed to change turnover rate of radioactive tracer dose in cows.

Anderson (1) found that TSR determinations in seven cows by substitution method averaged .46 mg/100 kg daily but with the radioactive thyroxine turnover method, TSR averaged only .31 mg/100 kg. Bauman et al. (2) showed when cows were given daily injections of 1-thyroxine at 125 or 150% of normal, turnover rates of tracer doses of radioactive thyroxine were increased from 26% a day to 38 and 50% a day. As PBI also increased by daily thyroxine injections, calculated TSR based on PBI were erroneous since they were two to three times the actual amount of thyroxine injected. Radio-thyroxine was metabolized in a different manner than the abnormally elevated PBI.

Plasma PBI may be increased many times over normal by feeding iodine supplements (7). Effect of PBI elevated by dietary iodine
upon TSR has not been investigated in the cow. A recent study of effect of dietary iodine for lactating cows upon TSR (11) found that different rates could be calculated according to whether PBI or plasma thyroxine was used for base amount, pool size, and turnover rates. Our study presents comparisons of TSR methods and evaluation of their significance.

Experimental Methods

**Experiment I.** The objective was to compare TSR determined by rates of disappearance of thyroxine $^{131}$I or PBI after injection of 1-thyroxine in same cows on low iodine and high iodine diets. Measured doses of thyroxine $^{131}$I were injected into the jugular vein of three mature Holstein cows in metabolism stalls. Blood was collected each day at 0800 and 1600 in heparinized tubes through 96 hr after dosing. Each cow was then injected intravenously with 20 mg 1-thyroxine. Blood collections were continued for 4 more days. Radioactivity of plasma was determined similarly to methods of Yousef and Johnson (13). Protein-bound iodine was determined in blood plasma taken for 2 days before 20 mg 1-thyroxine injections and for 4 following days by the alkaline ashing procedure of Brown et al. (3). Thyroxine secretion rates were calculated by methods of Yousef and Johnson (13) and Post and Mixner (9). Low iodine diet was alfalfa-grass mixed hay plus a concentrate mixture with plain (noniodized) salt. After completing tests on the low iodine diet, cows were fed the same diet supplemented with 100 mg iodine daily. The second series of tests was started 26 days after start of the iodine supplement.

**Experiment II.** Because TSR calculated on PBI from cows fed supplemental iodine seemed unreasonably high, disappearance of PBI from blood was compared with thyroxine $^{131}$I after 20 mg intravenous doses of 1-thyroxine. Eight Holstein cows were fed a low iodine control diet and seven were fed the same diet supplemented with 100 mg iodine from KI daily. Management, blood sampling, and analysis for these cows have been described (11). Thyroxine was determined by a modification of methods and materials of Abbott's Tetrasorb-125 kit$^1$. The same plasma was used for both PBI and thyroxine as trials showed that Tetrasorb-125 method gave comparable results from bovine serum and plasma. Plasma containing higher than normal thyroxine was diluted 1 to 5 rather than 1 to 2 for extraction, and separate extraction efficiencies were determined for each dilution. Thyroxine and PBI in plasma samples were determined four times at 2-mo intervals. Variance of rates of disappearance were analyzed to determine significance of effects of diet and method.

**Experiment III.** Results of Experiments I and II indicated that PBI, thyroxine $^{131}$I, and thyroxine I disappeared from blood at different rates and the differences were affected by iodine in diet and thyroxine in blood. These experiments were with the same cows at two different periods or with different groups of cows simultaneously. In Experiment III, simultaneous determinations of iodine disappearance rates from plasma were made in two trials in which PBI, thyroxine $^{131}$I, and thyroxine were analyzed after intravenous injections of 10 to 20 mg 1-thyroxine. In Trial 1, a polyethylene catheter was inserted into the jugular vein and thyroxine $^{131}$I dose was administered to each of five cows, two Jerseys and three Holsteins. Immediately after each dosing, 20 mg 1-thyroxine in alkaline aqueous solution was administered via the same catheter. Cows were kept in metabolism stalls. Blood samples for plasma were collected once daily 2 days before dosing and twice daily for five collections after dosing. In Trial 2 a similar test was made with five cows, except that thyroxine $^{131}$I was mixed with solution of 1-thyroxine about 30 min before dosing. Three Jerseys and two Holsteins were used and 1-thyroxine doses given were 16 mg for Jerseys and 20 mg for Holsteins. Methods of analysis were the same as in Experiments I and II.

**Results**

**Experiment I.** Disappearance rates of plasma $^{131}$I and PBI on low iodine and high iodine diet are in Fig. 1. Data are logarithmic means of observations for three cows for each diet. Hourly constants are indicated for each curve. Thyroxine $^{131}$I disappearance rate on high iodine diet before the 1-thyroxine dose was only 58% as fast as that on the low iodine diet. On the high iodine diet, a slowing of the PBI disappearance rate was also observed after 1-thyroxine injections. However, effects of the rate of $^{131}$I disappearance after thyroxine injection were reversed, and curves were different for the low and high iodine diets. On both diets the first samples after the 20 mg 1-thyroxine injection had dropped below the $^{131}$I trend line established earlier. Thereafter, on the low iodine diet a new uniform rate of decline was slower than both concurrent PBI and original

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**THYROXINE SECRETION**

![Graph showing the effect of low and high iodine diets on plasma thyroxine and PBI disappearance rates.](image)

**Fig. 1.** Comparison of effects of low iodine and high iodine diets on disappearance rates of plasma thyroxine$^{131}$I and PBI after intravenous injection of 20 mg 1-thyroxine.

**Table 1.** Variations in estimation of thyroxine secretion rates, based on logarithmic average rates of disappearance (turnover) curves in three experiments.

<table>
<thead>
<tr>
<th>Experiment and treatment or method</th>
<th>Normal plasma thyroxine</th>
<th>Thyroxine volume of distribution</th>
<th>Hourly rate of disappearance</th>
<th>Thyroxine secretion rate (mg/day)</th>
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<tbody>
<tr>
<td></td>
<td>(μg/liter)</td>
<td>(liter)</td>
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<td></td>
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<td><strong>Experiment I</strong></td>
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<td></td>
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<tr>
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<td>55.6</td>
<td>99.54</td>
<td>.0149</td>
<td>1.98</td>
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<tr>
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<td>41.84</td>
<td>.0164</td>
<td>.92</td>
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<td>101.32</td>
<td>.0117</td>
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<td>88.30</td>
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<td>65.30</td>
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<td>106.79</td>
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<td>68.7</td>
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<td>Trial 1 (Sequential)</td>
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<td>T$^{131}$I</td>
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* Protein-bound iodine.
131I rates. On the high iodine diet after 20 mg 1-thyroxine were injected, the 131I rate of disappearance nearly doubled for 2 days then returned to a rate similar to concurrent PBI and above original rate for 131I. Data for calculating TSR are in Table 1. Thyroxine distribution volume from PBI increase after 20 mg 1-thyroxine injection was about twice that calculated on tracer dose of thyroxine 131I indicating the tracer dose was not distributed in the same manner as the 20 mg dose. These differences, along with the difference in disappearance rate constants, resulted in TSR calculated on PBI about twice as high as those on T4 131I. Since the high iodine diet resulted in abnormally high PBI, when PBI was used to calculate normal plasma thyroxine it gave an erroneously high TSR.

**Experiment II.** Average rates of disappearance of plasma thyroxine I and PBI after intravenously injecting 20 mg 1-thyroxine on low iodine and supplemented diets are in Fig. 2. On each diet the hourly rate constants for thyroxine I were higher (P<.01) than those for PBI. Rates of disappearance of both PBI and thyroxine I were higher (P<.05) on low iodine diet than on supplemented diet, but differences between diets were less for thyroxine than PBI. Injecting 20 mg 1-thyroxine caused plasma thyroxine I to exceed PBI for 1 day on high iodine diet and 2 days on low iodine diet. Data for calculating TSR are in Table 1. Normal plasma levels and volume of distribution of thyroxine were higher on basis of PBI than thyroxine I on control diet and more than 50%
FIG. 3. Comparison of disappearance rates of thyroxine, thyroxine$^{131}$ I, and PBI after intravenous injection of 1-thyroxine following thyroxine$^{131}$ I (Trial 1) and mixed with thyroxine$^{131}$ I (Trial 2).

higher on high iodine diet. Thus, in spite of lower PBI rate constants, TSR calculated from PBI are 25 and 45% higher than those from thyroxine I.

Experiment III. Disappearance curves for thyroxine$^{131}$ I, thyroxine, and PBI are in Fig. 3. In Trial 1, when thyroxine$^{131}$ I was injected and followed by 20 mg 1-thyroxine, three significantly different curves resulted ($P<.01$). The rate constant for thyroxine$^{131}$ I was closer to that for PBI than for thyroxine.

A high percentage of thyroxine$^{131}$ I may have associated with PBI; when the massive 20 mg dose of 1-thyroxine came into the blood stream, however, it was not bound in the same manner. Thyroxine distribution volumes (Table 1), however, were intermediate on basis of thyroxine and much lower on basis of thyroxine$^{131}$ I than on PBI ($P<.01$).

In Trial 2 when the radioactive thyroxine was mixed with the large 1-thyroxine dose before injection, disappearance rate constants for the two were not significantly different ($P>.05$). But the calculated thyroxine distribution volumes were different ($P<.01$) as they were in Trial 1. This difference resulted in lower TSR estimations on basis of thyroxine$^{131}$ I than on thyroxine. In Trial 2 the PBI assayed lower than plasma thyroxine I, an unusual result with no logical explanation. For this reason, the TSR based on PBI seems abnormally low. If PBI in this trial were 100% T$_4$I, the
estimated TSR would be calculated as 1.34 mg/day making it the highest TSR in agreement with previous comparisons.

Discussion

Accuracy of TSR calculated from iodine disappearance rate constants is dependent upon the measured substance reacting as normal thyroxine secreted by the thyroid does. Vohnout et al. (12) analyzed $^{131}$I disappearance curves with 31 sheep over 96 hr after dosing. Analysis indicated that rate constants on plasma samples were affected by passage of thyroxine $^{131}$I in and out of extravascular spaces and by rate of thyroxine degradation and partial reutilization of released $^{131}$I. In their model they assumed that PBI and plasma thyroxine were the same; thus, incorporation of nonthyroxine $^{131}$I into PBI would introduce error in estimating thyroxine turnover. However, their model showed the disappearance rate of thyroxine $^{131}$I was 42% lower in 24 to 96 hr than in the first 48 hr. This is similar to our observation (Fig. 1) that on low iodine diets the rate of disappearance decreased with time and after injection of 20 mg thyroxine dose. These observations, however, disagree with those of Post and Mixner (9) who found no change in $^{131}$I disappearance rates. Although they didn’t give dietary information, the high PBI of their cows indicates the diet may have been supplemented with iodine causing the $^{131}$I rate constants before and after thyroxine injection and PBI rate constant to be similar. Freinkel and Lewis (4) produced a 240-hr uniform disappearance rate for radioactive thyroxine by feeding excess iodide to sheep.

Significantly different rate constants for disappearance of PBI and thyroxine in Experiment II indicate that plasma thyroxine cannot be represented precisely by PBI on low iodine diets and the difference between them increases when diets are supplemented with excessive iodine. On the low iodine diet thyroxine I was only 88% of PBI, and on the high iodine diet it was 59% (Table 1). The non-hormonal PBI turnover rate appears to be much slower than that of thyroxine I. Oppenheimer et al. (8) demonstrated that a rapidly exchangeable fraction of thyroxine occupied 35% of thyroxine space in humans compared to a slowly equilibrating fraction in 30% of thyroxine space. The increase above normal in thyroxine I after a 20 mg intravenous injection of $^{131}$I-thyroxine being greater than increase in PBI (Fig. 2 and 3) indicates that plasma is not able to bind all the massive thyroxine dose. Unbound thyroxine will be degraded or excreted faster than that normally bound in PBI.

Results of Experiment III confirm the inconsistency of plasma thyroxine and PBI under two different injection sequences. Radioactive thyroxine was representative of total thyroxine dose only when they were mixed before injection, confirming the application of exogenous isotope dilution technique. However, assuming that exogenous radioactive thyroxine is always representative of endogenous thyroxine metabolism may be mistaken, especially if unusual plasma iodine or thyroxine binding conditions exist.

Results of these experiments indicate that true thyroxine disappearance rates, assumed to equal TSR, can be approximated only when a minimum tracer dose of radioactive thyroxine is used to obtain disappearance constant and distribution volume and actual plasma thyroxine is determined to establish normal blood levels. Amount of radioactive thyroxine required to obtain suitable plasma activity from a 500 kg cow should be about 100 μcurie (Ci). If the activity of dosing thyroxine is 50 μCi/g, the dose would contain 2 μg. At a TSR of 1.44 mg thyroxine daily, endogenous thyroxine would be 1 μg/min so the tracer dose would equal 2 min thyroid output, still slightly above physiological levels. The method described by Yousef and Johnson (13), with the exception that plasma thyroxine determinations be made in place of PBI, should be accurate for normal low iodine diets. Conditions altering plasma binding of iodine or thyroxine can be expected to produce mistaken TSR because they also alter distribution volume and disappearance rate constant of the tracer dose.

References


