Dependence of thyroxine utilization rate on dietary composition

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1. The rate of utilization (k) of labelled thyroxine increased when energy intake, given as standard pig meal, was doubled from 20 to 40 g/kg body-weight per d. When the bulk of food was increased, but not its energy content, the value of k did not change. Ambient temperature was constant throughout the experiment.

2. Groups of pigs were given pig meal at 20 g/kg body-weight, 40 g/kg body-weight or 20 g/kg body-weight plus a supplement. The supplement was of equivalent energy content to 20 g pig meal/kg body-weight and consisted of coconut (high-fat), fish meal (high-protein) or glucose. The values of k were similar on diets (g/kg body-weight) of 40 pig meal, 20 pig meal plus coconut, and 20 pig meal plus fish meal. When the supplement was glucose however the values of k were similar to that for 20 g pig meal/kg body-weight.

3. The plasma concentrations of T_4, and triiodothyronine were not affected by eating a meal, or by changing the energy intake presented as pig meal. A comparison between pigs given 20 g pig meal/kg body-weight plus supplements of bran, coconut, fish meal or glucose revealed differences in the concentration of both hormones. When food was withdrawn for 5 d the concentrations of both hormones declined.

A major factor influencing the rate of secretion and utilization of thyroid hormones in animals is a change in ambient temperature (Freinkel & Lewis, 1957; Reichlin, 1966). It has been demonstrated that an increase in heat loss from an animal is followed by the release of thyroid-stimulating hormone and a subsequent increase in the secretion of thyroxine (T_4), (Hershman *et al.* 1970; Reichlin *et al.* 1972). Exposure to a cold ambient temperature, however, is usually accompanied by an increase in food intake. Evans & Ingram (1977) found that although exposure to cold initiated an increase in the secretion of T_4, the rate of utilization of T_4 remained unchanged unless food intake was increased. Subsequently Ingram & Kaciuba-Uscilko (1977) reported that, in growing pigs, changes as great as those associated with the combination of a fall in ambient temperature and an increase in energy intake, could be elicited by a similar increase in energy intake alone.

The next steps were to determine: (1) whether the stimulus to an increased rate of use of T_4 was a simple increase in energy intake, or if the quantity of specific nutrients needed to be increased; (2) whether the change in the rate of use of T_4 associated with an increase in energy intake was accompanied by a change in the plasma levels of T_4 and triiodothyronine (T_3). In the present study these points have been investigated in young, immature pigs. A preliminary communication of part of these results has been presented (Ingram & Dauncey, 1980).

MATERIALS AND METHODS

Animals

Sixty-eight pigs of the Large White breed aged between 2 and 3 months were used.

Housing

Each pig was housed separately in a cage at a controlled environmental temperature of 25° with continuous lighting.

Table 1. *Composition of foods (g/kg) and supplements given to pigs*

Food	Gross Energy (kJ)	Available nutrients		
		Protein	Fat	Carbohydrate
Pig meal	15800	180	20	620
Coconut	28500	60	620	60
Fish meal	17900	660	60	0
Glucose	15600	0	0	1000
Bran*	8720	140	60	270

* Bran remains mostly undigested in the pig.

Feeding and diet

The pigs were fed once daily between 08.00 and 10.00 hours. Water was provided *ad lib.* The amount of food given was related to the animal's body-weight and varied between 10 and 60 g/kg body-weight according to the experiment. Under normal husbandry conditions pigs are fed at a rate of 40 or 45 g/kg body-weight. The standard diet consisted of a commercially-available pig meal, and in addition some animals were given a supplement of bran, fish meal, desiccated coconut, or glucose. The compositions of all foods as derived from food tables (Paul & Southgate, 1978) or maker's analysis are given in Table 1.

Surgery

All pigs had a catheter inserted into the jugular vein. The catheter which was led under the skin and emerged between the shoulder blades, was plugged, coiled and kept in a pouch on the pig's back. Operations were carried out under general anaesthetic and sterile conditions 4 to 5 d before the experiment began.

Blood samples

Samples of blood were taken while the pigs were in their normal cages. The blood was placed in a heparinized tube, mixed and the plasma separated by centrifugation. The plasma concentrations of hormones were determined in separate animals from those used for the injection of labelled hormone.

Rate of utilization of T_4

The rate of utilization of T_4 was estimated as previously described (Evans & Ingram, 1977; Ingram & Kaciuba-Uscilko, 1977). Thyroxine labelled with [125]I (The Radiochemical Centre, Amersham, Bucks.) was injected in a dose of 25 μCi. Samples were then taken every 3–12 h for 70 h beginning 12 h after the injection. The radioactive content was expressed as the percentage of the dose initially injected/1 plasma. The dose administered was calculated from the ^{125}I estimated in a 1:999 dilution of the solution injected. As in previous studies the labelled hormone was washed out of the catheter by repeated withdrawal and reinjection of blood.

Analysis of results

The fractional disappearance rate constant k was calculated using a single exponential decay model:

$$y = be^{-kx},$$

where y is the % dose of [125]I /ml plasma, x is the time after injection (h), b is the range of % dose ^{125}I as time passes from zero to infinity, k is the ^{125}I disappearance rate constant. One value of k was thus obtained from each curve.

Estimation of serum T_3 and T_4

The plasma was separated by centrifugation and the samples stored at $-20°$ before being dispatched to the hospital laboratory for analysis. The concentration of T_4 was determined using a radioimmunoassay technique previously described (Evans *et al.* 1977). Values were compatible with values previously obtained using competitive binding assays (Evans & Ingram 1974, 1977). Serum T_3 concentration was determined using a solid phase radioimmunoassay technique (Immophase, Corning Medical, Halstead, Essex).

All assays were carried out in duplicate and subjected to routine quality-control procedures and variation between assays was indicated by 95 % confidence limits of 6 % for T_4 and 9 % for T_3. In any instance where a value was believed to be abnormally high or low a second analysis was made.

Experimental procedure

All pigs were kept at the Institute of Animal Physiology, Babraham, on a diet of standard pig, meal fed at a quantity equivalent to 20 g/kg body-weight for 1 week before the experiments started. The order in which different diets were presented was varied between pigs. In the determination of k the pigs were fed the given diet for 5 d before injection of ^{125}I thyroxine and the diet continued while k was being determined.

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RESULTS

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The rate of utilization of T_4

Four groups of pigs all received 20 g pig meal/kg body-weight for 1 week and 40 g/kg body-weight in a second week. In a third week each group received a different supplement of bran, fish meal, glucose, or desiccated coconut in addition to 20 g pig meal/kg body-weight. The supplement of bran was equivalent in bulk to 20 g pig meal/kg body-weight. The remaining supplements were equivalent in energy content to 20 g pig meal/kg body-weight. The results are given in Table 2.

Effect of energy intake. The effect of changing the energy intake from 20 to 40 g/kg body-weight can be evaluated from the results obtained on thirty-one pigs. The mean value for k, which indicates the rate of utilization, was 0·0296 and 0·0339 for 20 and 40 g/kg body-weight respectively. A paired t test indicated that there was a statistically-significant difference between these values ($P < 0.001$).

Effect of nutrient composition. Comparisons between 40 g/kg body-weight and 20 g/kg body-weight plus glucose, fish meal or coconut were also made by paired t tests. There was no significant difference ($P > 0.05$) in the value of k for 40 g/kg body-weight and 20 g/kg body-weight plus coconut (high-fat) or 20 g/kg body-weight plus fish meal (high-protein). The rate of utilization was however significantly greater ($P < 0.02$) on 40 g pig meal/kg body-weight than on 20 g/kg body-weight plus glucose. On the other hand the values of k for 20 g/kg body-weight alone and supplemented with glucose were not different ($P > 0.05$); while there were significant differences between 20 g pig meal/kg body-weight alone and supplemented with coconut or fish meal ($P < 0.02$).

Effect of bulk of food. The effect of changing the bulk of the food but not its energy content could be determined from the study using a supplement of bran. The value of k was not significantly different ($P > 0.05$) between 20 g pig meal/kg alone and 20 g pig meal/kg supplemented with bran; but it was different between 40 g pig meal/kg and 20 g pig meal/kg plus bran ($P < 0.02$) indicating that the bulk of food did not change the value of k.

Table 2. *Values of rate of utilization for thyroxine for pigs given different diets**

(Mean values with their standard errors; no. of pigs in parentheses)

Dietary supplement Diet (g/kg body -weight)	20 + supplement		20		40	
	Mean	SEM	Mean	SEM	Mean	SEM
Bran (9)	0·0305	0·0014	0·0321	0·0009	0·0338	0·0009
Fish meal (8)	0·0349	0·0012	0·0312	0·0014	0·0362	0·0013
Coconut (7)	0·0369	0·0027	0·0262	0·0004	0·0338	0·0027
Glucose (7)	0·0271	0·0011	0·0282	0·0013	0·0361	0·0042

*For details, see Table 1.

Concentrations of T_3 and T_4 in plasma

The day to day variations in the concentrations of T_4 in plasma were sometimes as great as 10 nmol/l. The mean values for individual animals also varied and for this reason comparisons of T_3 and T_4 concentrations were restricted to those within individuals using paired t tests. Variations in T_3 concentration were smaller than for T_4 but inspection of the records suggested that a rise or fall in T_4 was accompanied by a corresponding change in T_3 and this was confirmed by a statistically-significant correlation coefficient ($P < 0·01$).

Time of feeding. The possible effects of taking blood samples before or after a meal were investigated in two sets of pigs all fed at the same time of day. Four pigs were fed at 30 g/kg body-weight as pig meal once daily for 1 week and 60 g/kg body-weight for the next week. Another four pigs were given 10 and 40 g/kg body-weight in successive weeks. Blood samples were taken over the last 5 d of each diet, one sample just before feeding and the next sample 2 h later. The differences in serum concentrations of T_3 and T_4 displayed no consistent trends and no statistically-significant differences were found (Fig. 1).

Energy intake. The effect of energy intake on values of serum T_3 and T_4 were determined in two experiments using standard pig meal. In the first experiment fourteen pigs were given 20 g/kg body-weight per d for 1 week and then 40 g/kg for the next week. The values for each hormone were then determined from the mean values for each pig over the last 5 d. This provided a comparison between the lowest intake which would just allow growth and an intake which allowed moderate growth at a rate typical of husbandry conditions. In the second experiment seven pigs were fed at 15 g/kg body-weight, which was just enough to prevent loss of weight for 1 week, and then food was withheld for 5 d. Hormone levels were obtained from the values over the last 5 d on 15 g/kg body-weight and over the entire 5 d in which food was withheld. The results of both experiments are shown in Table 3. Paired t tests within individual pigs revealed no differences in hormone levels between food intakes at 20 and 40 g/kg body-weight, but similar tests between intakes of 15 and og/kg body-weight of food were statistically significant for both T_3 and T_4 ($P < 0·001$, and $P = 0·02-0·01$ respectively). As can be seen the values for 15, 20 and 40 g/kg body-weight for particular hormones were all similar.

Composition of the diet. The possible effects of the composition of the diet on the values of T_3 and T_4 were investigated in a series of studies using four different groups of five or six pigs for each diet. The animals were given 20 g pig meal/kg body-weight plus the various supplements and the values for T_3 and T_4 were again determined over the last 5 d. As in the previous experiments the supplement which consisted of the energy equivalent to 20 g/kg body-weight was given as fish meal (high-protein), desiccated coconut (high-fat) or glucose. In addition one group received a supplement of bran which was equivalent in bulk to 20 g pig meal/kg body-weight. The results which are given in Table 4 were subjected to the
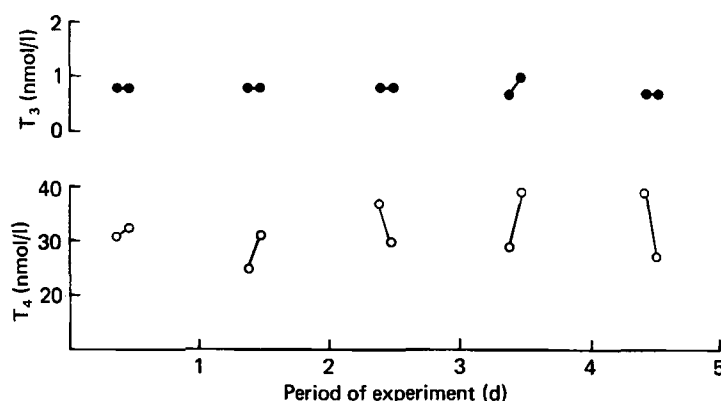
Fig. 1. Serum concentrations of thyroid hormones (T_4 and T_3; nmol/l) in samples of blood taken just before feeding and 2 h later over a 5 d period. Results from a single pig given 40 g/kg body-weight. (For details, see p. 528 and Table 1.)

Table 3. *Effect of energy intake given as pig meal* on values of plasma thyroid hormones (T_3 and T_4)*

(Mean values with their standard errors; in Expt 1 the pigs were growing, in Expt 2 they were not; no. of pigs in parentheses)

Expt no.	Food intake (g/kg body-weight)	T_3 Mean	T_3 SEM	T_4 Mean	T_4 SEM
1 (14)	20	1·7	0·19	37·1	1·9
	40	1·9	0·18	35·1	1·9
2 (7)	15	1·3	0·12	37·7	15·6
	0	0·7	0·11	22·3	4·2

Table 4. *Plasma thyroid hormones (T_3 and T_4) concentrations (nmol/l) in pigs given 20 g pig meal/kg body-weight per d supplemented with glucose, coconut or fish meal**

(Mean values with their standard errors. The diets had the same energy content as a diet of 40 g pig meal/kg body-weight, the diet supplement with bran had the same bulk as the 40 g/kg body-weight and the same energy content as 20 g pig meal/kg body-weight)

Supplement...	Bran Mean	Bran SEM	Fish meal Mean	Fish meal SEM	Coconut Mean	Coconut SEM	Glucose Mean	Glucose SEM
T_3	1·30	0·15	0·67	0·06	1·14	0·05	2·30	0.18
T_4	30·3	1·7	44·8	1·5	38·8	1·7	38·5	2·7

* For details, see Table 1.

analysis of variance which revealed a statistically-significant effect for both T_3 and T_4 ($P < 0.001$ and $P < 0.01-0.001$ respectively). The value for T_3 was significantly lower for the diet containing fish meal than for glucose. The concentration of T_4 was significantly lower on the diet containing bran than on fish meal.

DISCUSSION

Rats which are exposed to cold and allowed to eat more food have an increased loss of T_4 in the faeces (Van Middlesworth, 1960). Changes in diet have in fact been shown to have a greater effect on faecal excretion of T_4 than cold alone (Hillier, 1968; Straw, 1969). Increased faecal loss of T_4 appears to be related to a greater enterohepatic circulation of thyroxine (Cottle & Veress, 1966) and it has been suggested that the increased faecal bulk augments the loss of T_4 in the faeces and so causes an increased rate of turnover of the hormone. In the present study the increased value of k when food intake increased from 20 to 40 g/kg body-weight might have been due to the excretion of T_4 in the faeces. This was unlikely to have been the situation since when the pigs were given bran, which is not digested, the bulk of faeces would have been increased more than when changing from 20 to 40 g pig meal/kg body-weight; while the value of k did not alter. A similar result was obtained previously (Ingram & Kaciuba-Uscilko, 1977) when the bulk of the diet was increased with chopped straw. The diet containing straw was not, however, readily eaten and it was possible that the unappetizing food could have influenced the secretion or metabolism of T_4. By contrast the bran was eaten much more readily. For these reasons the differences in the value of k on diets of 20 g pig meal/kg body-weight plus a supplement seem unlikely to have been related to faecal bulk even though there was a marked difference in the amount of dietary fibre in, for example, the diets containing desiccated coconut, or glucose. Moreover, in rats which have been exposed to the cold it has also been shown that the high secretion rate of T_4 is independent of the bulk of the diet (Heroux & Petrovic, 1969). The pigs given a supplement of bran did have a low value for plasma T_4 which may have been related to faecal loss of hormone.

The effects of dietary composition on metabolic rate between 12 and 20 h after feeding have been studied by Dauncey & Ingram (1979). The changes they found in metabolic rate correspond closely to the changes in the rate of utilization of T_4 observed in the present study. Increasing the energy intake as pig meal, coconut or fish meal resulted in an increase in metabolic rate during the whole 8 h period, but when the extra energy was supplied as glucose the metabolic rate was unchanged. It appears therefore that specific nutrients can influence both the rate of utilization of T_4 and the resting metabolic rate. The studies from which this conclusion was reached did however involve diets in which particular nutrients accounted for a large proportion of the total energy intake. In a more balanced diet the effects of specific nutrients would probably be less obvious.

There are two explanations for the changes in the rate of utilization of T_4. One is that the production of T_4 is stimulated by some constituents in food either directly or indirectly and this increases metabolic rate. If this was the situation then it would follow that glucose is not a sufficient stimulant and that utilization of T_4 parallels its secretion. Another possibility is that there is an increased use of T_4 caused by the processing of complex foods which is itself sufficient to initiate and maintain a high rate of metabolism. In this event the increased consumption of T_4 would lead to an increased rate of secretion through the normal feedback control, and so the value of k would rise. The fish meal may have contained thyroid hormones and TSH and these would have exerted a separate effect from that related to the energy content of the diet. It would appear, however, that even if these hormones were present in the fish meal the effect that they had on the utilization of T_4 was small since the value of k was similar for 60 g pig meal/kg body-weight and for the diet of 20 g pig meal/kg body-weight plus the supplement of fish meal. The slightly higher values for plasma T_4 after fish meal may on the other hand have been related to ingested hormone.

If the increased turnover of T_4 stems from an increased metabolic rate then initially it would be expected that the T_4 levels would decline. In the present study T_4 was estimated only after the change of diet had been established for 2 d and so a fall would not in any event

have been seen. Statistically-significant changes in the concentration of thyroid hormones were seen when food was withheld completely. The fall in T_3 has been observed in man where it was associated with a rise in reverse T_3, but in these studies the levels of T_4 declined only slightly and in some cases did not change (Portnay *et al.* 1974; Chopra & Smith, 1975; Vagenakis *et al.* 1975; Palmblad *et al.* 1977). The fact that the plasma level of T_4 declined when food was withheld is pertinent to the present discussion since it indicates that a simple fall in T_4 is not sufficient to increase the rate of secretion. In fact it tends to support the usual view that metabolic rate is determined by T_4 level, rather that the utilization rate of T_4 being determined by the metabolic rate. The effect of dietary supplements on serum T_3 needs further investigation involving measurements of reverse T_3. It may however be of significance that the lowest value for T_3 was associated with the most rapid rate of utilization of T_4 and the highest value with the lowest rate.

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