

Effects of thyroid status on insulin-like growth factor-I, growth hormone and insulin are modified by food intake

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Abstract

Understanding the interactions between metabolic signals that regulate insulin-like growth factor-I (IGF-I) is crucial to a recognition of mechanisms that control mammalian growth. Thyroid hormones (THs) are essential for normal growth and development, and it has been suggested previously that they can modify circulating IGF-I concentrations. However, the fact that THs influence food intake, which can itself affect plasma IGF-I levels, has been ignored in previous studies. We have therefore investigated the effects of thyroid status on plasma IGF-I under conditions of controlled food intake in young growing pigs. Circulating IGF-I, growth hormone (GH) and insulin levels, were studied in hypo- and hyperthyroid animals on the same level of food intake as euthyroid controls. In addition, a separate group of hyperthyroid animals was given double the amount of food, in order to assess the influence of increased food intake, as would occur naturally in the hyperthyroid state. Hypothyroid animals and hyperthyroids with extra food had the greatest increase in body weight over the 3 weeks of treatment. These two groups had significantly higher circulating IGF-I and insulin concentrations than either the euthyroid or hyperthyroid animals on the same food intake. Integration of GH concentrations from samples taken every 20 min over a 9 h period showed that, by contrast with IGF-I and insulin levels, GH levels were significantly lower in hypothyroids and hyperthyroids on extra food compared with the euthyroids and the hyperthyroids on the same food intake. We conclude that the effects of thyroid status on IGF-I are mediated in part by the effects that THs have on energy balance, and that nutritional signals are capable of modifying the influence of thyroid status *per se* on circulating IGF-I concentrations.

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Introduction

There are close interrelations between the multitude of hormones that regulate mammalian growth. Insulin-like growth factor-I (IGF-I), one of the key mediators of cellular metabolism and proliferation, is directly influenced by growth hormone (GH) (1, 2). Thyroid hormones (THs) can also modify plasma IGF-I levels, and a direct correlation between circulating levels of IGF-I and THs has been reported (3–5). The effects of THs on IGF-I may be mediated by stimulation of GH secretion, as has been demonstrated in pituitary tumour cell lines *in vitro* (6, 7) and normal thyrotrophs *in vivo* (8, 9). However, administration of THs can also increase circulating IGF-I concentrations independently of GH, as has been observed in hypophysectomised rats (10) and other animal models (11–12).

The above observations do not generally take account of the regulatory effects of nutritional status on the hormonal axes described. A direct correlation exists between energy intake and circulating levels of THs

(13, 14) and IGF-I (15–17), whereas undernutrition leads to an elevation in circulating GH (18). The precise mechanisms by which energy intake modifies circulating IGF-I concentrations are unclear, although they may involve changes in circulating thyroid and pancreatic hormone levels (19). Direct assessment of the effects of thyroid status on IGF-I is difficult, because THs themselves influence energy expenditure and voluntary food intake (20). Thus, THs have the capacity to affect IGF-I levels indirectly by their effect on energy balance. This factor has been neglected in most studies, in which circulating IGF-I levels in response to changes in thyroid status have been assessed in animal models or human subjects that have free access to food.

Our aim, therefore, has been to study the effects of thyroid status on circulating IGF-I levels under conditions of controlled energy intake. Since the influence of nutrition on the hormones involved in growth may be particularly significant during the period of rapid growth and development in early postnatal life, we have used young growing pigs for our study. We

have compared circulating IGF-I concentrations in hypothyroid, euthyroid control, and hyperthyroid animals that have been kept on the same level of food intake. In order to mimic the hyperthyroid state, in which food intake is voluntarily increased, a second hyperthyroid group of animals given extra food was also included. GH, which plays a crucial role in modulating plasma IGF-I concentrations, has been measured in the different treatment groups. We have also studied changes in circulating insulin concentrations, because plasma insulin levels are regulated independently of IGF-I by food intake, and the two hormones share pathways that exert similar effects on cellular metabolism.

Materials and methods

Animals and procedures

Male pigs of the Large White breed were used for all the studies. The animals were weaned at 3 weeks of age, and housed singly at a temperature of 26 °C, which is close to thermal neutrality. They were fed a standard diet (UltraWean; Dalgety, Bristol, Avon, UK) at a set time each day. The feed contained 14 kJ gross energy/g wet weight, and consisted of 32.0% carbohydrate, 22.0% protein, 5.5% oil, 3.5% fibre, 6.0% ash, and added minerals and vitamins. Water was freely available, and lights were on from 0900 to 2100 h.

Thyroidectomies were carried out under general halothane anaesthesia (Fluothane, ICI Pharmaceuticals, Macclesfield, Cheshire, UK) and sterile conditions, following sedation with ketamine hydrochloride (1.0 ml Vetalar 100 mg/ml; Parke-Davis Veterinary, Pontypool, Gwent, UK) given intramuscularly. To enable sampling of blood at frequent intervals with minimum disturbance to them, the animals were catheterised. The pig has no obvious superficial vein, and therefore the catheter was inserted into the jugular vein under general anaesthesia as described above. Blood samples were taken through the indwelling catheter into heparinised tubes, and after separation plasma fractions were stored at -40 °C until assayed for concentrations of hormones. All procedures were carried out with full authorisation from the UK Home Office.

Treatments

Five groups each of four littermate pigs were investigated. The animals were fed 30 g standard feed/kg body weight per day (3%). After 1 week, three animals from each litter were thyroidectomised and placed on 2 mg methimazole (Sigma, Poole, Dorset, UK) per kg body weight per day, to prevent regeneration of the remnants of the thyroid gland. One of these animals was maintained hypothyroid, while the other two received thyroxine (T₄; Sigma), the dose of which was gradually increased to 20 µg/kg body weight per day by 1 week

after thyroidectomy. This dose of T₄ was selected to give supraphysiological circulating levels of T₄ without rendering the animal grossly hyperthyroid, and was chosen according to a previously estimated distribution volume of approximately 2 l (14) and a half-life of nearly 1 day for T₄ (21) in animals of the same breed and similar age. For one of these hyperthyroid animals, the food given was increased to 60 g/kg body weight per day (6%) after thyroidectomy. The doses of methimazole and T₄ were given orally with the feed. The fourth animal in each litter was kept, without any surgical manipulation, as a euthyroid control on 3% feed. Hence each litter consisted of a hypothyroid, a euthyroid and a hyperthyroid animal, each on 3% food intake, and a hyperthyroid animal on 6% feed. The treatment regimes were maintained for 3 weeks, after which the animals were catheterised as described above. Blood sampling was then carried out after a period of 5 days, in order to circumvent any acute effects of anaesthesia and operational procedures on circulating hormone levels. A total of 37 samples were collected during a 24 h period. Samples (3–4 ml) were taken every 20 min during the day (0900–1830 h), and every 1–2 h thereafter.

Plasma thyroid hormone measurement

Total T₄ and total tri-iodothyronine (T₃) concentrations were measured using commercially available Coat-a-Count kits (Diagnostic Products Corporation, Los Angeles, CA, USA), which incorporate a solid phase RIA system. All samples from littermate animals were analysed in the same batch. The intra-assay coefficients of variation for T₄ and T₃ measurements were less than 3.8% and less than 8.9% respectively.

Plasma IGF-I measurement

Human and porcine IGF-I have identical amino acid sequences (22, 23). Therefore, IGF-I was measured in plasma by an RIA method in use for measurement of IGF-I in human serum. The samples were measured in duplicate following acid-ethanol extraction of the hormone from the protein-bound pool as described previously (24). This method was shown to yield a good recovery of plasma IGF-I, and previous studies in pigs with widely varying circulating concentrations of IGF-I-binding proteins have shown no interference in the assay from IGF-binding proteins (25, 26). IGF-I for calibrants and radiolabelling was obtained from Bachem (UK) Ltd (Saffron Walden, Essex, UK). The antiserum (NIDDK; National Hormone and Pituitary Programmes, Baltimore, MD, USA) was rabbit antibody for human IGF-I. Bound and free fractions were separated by using sheep anti-rabbit antibodies. All samples from littermate animals were analysed in the same batch. The intra-assay coefficients of variation for the plasma IGF-I assay were less than 7.5%.

Plasma GH measurement

GH in plasma samples was measured in duplicate by an in-house RIA that used anti-porcine GH antibodies that were raised in guinea pigs and were kind donations of Dr H L Buttle. GH for radiolabelling by the Iodo-Gen method (Pierce Chemical Co., Rockfield, IL, USA) was recombinant-derived porcine GH, and was a donation from Monsanto Co., St Louis, MO, USA. The GH for the calibrants was pituitary-derived porcine GH (NIDDK; National Hormone and Pituitary Programmes). Donkey anti-guinea pig serum was used for separation of bound and free fractions. All samples from littermate animals were analysed in the same batch, and the intra-assay coefficients of variation were less than 7.8%.

Plasma insulin measurement

Insulin in plasma samples was measured by an in-house RIA that used anti-porcine insulin antibodies that were raised in guinea pig. Insulin for assay calibrants and radiolabelling was purchased from Sigma. All samples from littermate animals were analysed in the same batch, and the intra-assay coefficients of variation were less than 6.5%.

Statistical analysis

All comparisons were made by analysis of variance, followed by a multiple comparison test using Studentised Range.

Results

Growth rates

Animals had comparable body weights at the start of the treatment period, and the overall value was 6.9 ± 0.2 kg (mean \pm S.E.M.). The rates of change in body weights of the four treatment groups are given in Table 1. The hypothyroid animals had a faster rate of

increase in body weight than euthyroid controls ($P < 0.05$). However, the greatest rate of increase in weight was observed in the hyperthyroid animals on 6% food intake ($P < 0.01$ for all comparisons). This group weighed on average 40–50% more than either the euthyroid or the hyperthyroid animals on 3% food intake. These body weights, however, do not allow strict comparisons between rates of lean body growth in the different groups, because of differences in the efficiency of energy utilisation, body composition and linear growth, which are known to be modified by thyroid status.

Plasma thyroid hormones

Hypothyroid animals had circulating T_4 concentrations that were below the detection limit of the assay (<3 nmol/l). Because T_4 was administered orally in the feed, plasma T_4 was measured in four samples collected over the 17.5–23.5 h period after presentation of food. The half-life of T_4 in the pig is approximately 1 day (21), and the present values are therefore near-trough levels of the hormone concentrations in these animals. The mean total T_4 concentrations for the different treatment groups over this time-period are given in Table 1. The hyperthyroid animals on 3% and 6% food intakes had circulating T_4 levels which were on average 125% and 87% higher than the euthyroid control group. These trough levels of T_4 in the hyperthyroid animals were at least 40% higher than the physiologically elevated levels observed in animals of the same breed and age on a high food intake or after acclimation to a cold ambient temperature (14, 27).

Table 1 also gives the circulating T_3 concentrations in the four groups of animals. Since circulating T_3 levels rise rapidly after food intake in the young pig (28), the values are those from samples taken at time points before presentation of food and treatment with T_4 . Hypothyroid animals had circulating T_3 levels that were at all times below the detection limit of the assay (<1 nmol/l). However, no difference was observed between T_3 concentrations in the euthyroid and the

Table 1 Body weights and plasma concentrations of thyroid hormones in the four treatment groups. Mean \pm S.E.M. for five animals in each group.

Treatment group	Food intake (%)	Body weight at start (kg)	Body weight at end (kg)	Increase in weight (%)	Plasma T_4 (nmol/l)	Plasma T_3 (nmol/l)
Hypothyroid	3	7.0 ± 0.5	14.6 ± 1.0	110.1 ± 4.6	N.D.	N.D.
Euthyroid	3	7.1 ± 0.5	12.5 ± 1.0	75.6 ± 4.5	37.9 ± 3.9	0.77 ± 0.50
Hyperthyroid	3	7.3 ± 0.5	13.4 ± 1.1	81.6 ± 7.8	85.2 ± 11.0	0.88 ± 0.21
Hyperthyroid	6	6.4 ± 0.3	18.4 ± 1.3	187.2 ± 12.7	70.8 ± 9.7	0.86 ± 0.14

Hypothyroid and hyperthyroid animals had been thyroidectomised, and received 2 mg methimazole/kg body weight per day. Hyperthyroid animals received, in addition, T_4 at a dose of 20 μ g/kg body weight per day. Food intakes were either 30 or 60 g feed/kg body weight per day (3% or 6% respectively). The start of the treatment regimes was taken as the day of thyroidectomy, after which food intakes and thyroid status were manipulated. The treatment regimes were maintained for 4 weeks. Plasma thyroid hormone levels are those at the end of this period, when animals were 8 weeks old. N.D. = not detected; below the detection limit of the assay (<3.0 nmol/l for T_4 and <0.2 nmol/l for T_3).

hyperthyroid groups. With the half-life of T_3 of approximately 1 h in the pig (21), these trough levels of T_3 suggest that at no time were the plasma T_3 levels in the hyperthyroid group below those in the euthyroid animals.

Plasma GH levels

Figure 1 depicts the GH levels in one littermate treatment group during the 24 h blood sampling period. Due to the inherently pulsatile nature of GH secretion, comparisons between GH levels in different litters were made between the normalised areas under respective GH curves plotted against time. The results for the integrated GH areas, given in Table 2, revealed significant differences between the four treatment groups ($P < 0.01$). Compared with the euthyroid controls, the GH values were significantly lower in the

hypothyroid animals on the same level of food intake and the hyperthyroids on extra food ($P < 0.05$ for both comparisons). The areas under the GH curves for hyperthyroid animals on 3% food intake were not different from those for the euthyroids, but were greater than those for the hypothyroids on the same food intake and the hyperthyroids on extra food ($P < 0.05$ for both comparisons).

In order to assess differences in the baseline GH levels and the size and number of GH peaks, we adopted a set of simple criteria for defining a GH peak. A peak was defined as an increase of greater than 4 ng/ml in GH level over at least one of the preceding two values, or an increase of greater than 5 ng/ml over one of the preceding three values (this was to allow for peaks with a slow increase in GH levels). The beginning of each peak was defined as the time point after which there was an immediate increase of 2 ng/ml or greater in GH value. The end of a peak was defined as the time

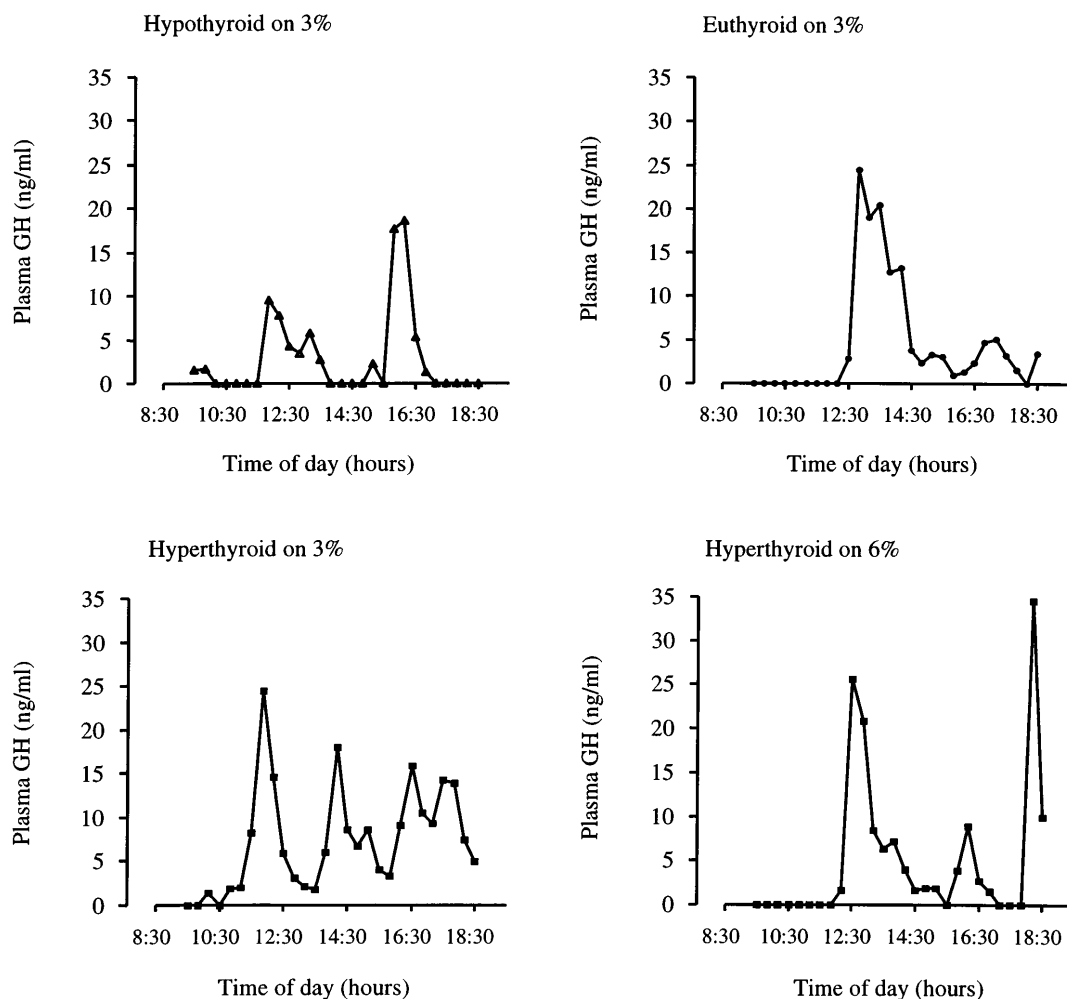


Figure 1 Plasma GH concentrations during a 9-h period of sampling in the 4 treatment groups. Animals were fed at 1600 h each day, including the day when the blood samples were taken. The figures present GH levels in one representative group of litter mate animals.

Table 2 Plasma GH data obtained from the four groups of animals after the 4 weeks of treatment. Mean \pm s.e.m. from five animals in each group.

Treatment group	Food intake (%)	Total GH area (arbitrary units)	Number of GH peaks	GH peak height (ng/ml)	Baseline GH level (ng/ml)
Hypothyroid	3	0.47 \pm 0.03 ^a	1.6 \pm 0.24	18.0 \pm 2.98	0.74 \pm 0.23
Euthyroid	3	1.00 \pm 0.15	2.8 \pm 0.37	14.0 \pm 1.85	1.12 \pm 0.39
Hyperthyroid	3	1.05 \pm 0.12	2.8 \pm 0.48	16.7 \pm 1.41	1.57 \pm 0.72
Hyperthyroid	6	0.63 \pm 0.07 ^a	3.0 \pm 0.00 ^b	15.9 \pm 2.68	0.82 \pm 0.29

Hypothyroid and hyperthyroid animals had been thyroidectomised, and received methimazole. Hyperthyroid animals received, in addition, T₄ at a dose of 20 μ g/kg body weight per day. Food intakes are given as percentage of body weight. Plasma GH was measured in a total of 28 samples taken during a 9 h period. ^a $P < 0.05$ compared with euthyroid and hyperthyroid on 3% food intake; ^b $P < 0.05$ compared with hypothyroid on 3% food intake.

point at which there was no subsequent decrease of greater than 2 ng/ml in GH value before a subsequent peak or before the end of the sampling period. According to these criteria, there were no differences in baseline (between-peak) GH levels or peak heights between different treatment groups (Table 2). However, the mean number of GH peaks was higher in the hyperthyroids on 6% food compared with hypothyroid animals ($P < 0.05$).

Plasma IGF-I levels

For each treatment group, the changes in the mean plasma IGF-I concentration over a 24 h period are depicted in Fig. 2. The mean IGF-I value for each animal was determined by averaging the values from samples

taken every 2 h throughout the 24 h period. These were used to establish a group mean for each treatment, and are given in Fig. 3. There was a significant difference as a result of treatment ($P < 0.0001$) between different groups. Further statistical analysis showed that both the hypothyroid animals on 3% food intake and the hyperthyroids on extra food had higher circulating IGF-I concentrations than the euthyroid controls and the hyperthyroids on 3% food intake ($P < 0.01$ for all comparisons).

Plasma insulin levels

Figure 4 shows the plasma insulin concentrations in one group of littermate animals during the 24 h period of blood sampling. Insulin values from the 37 samples

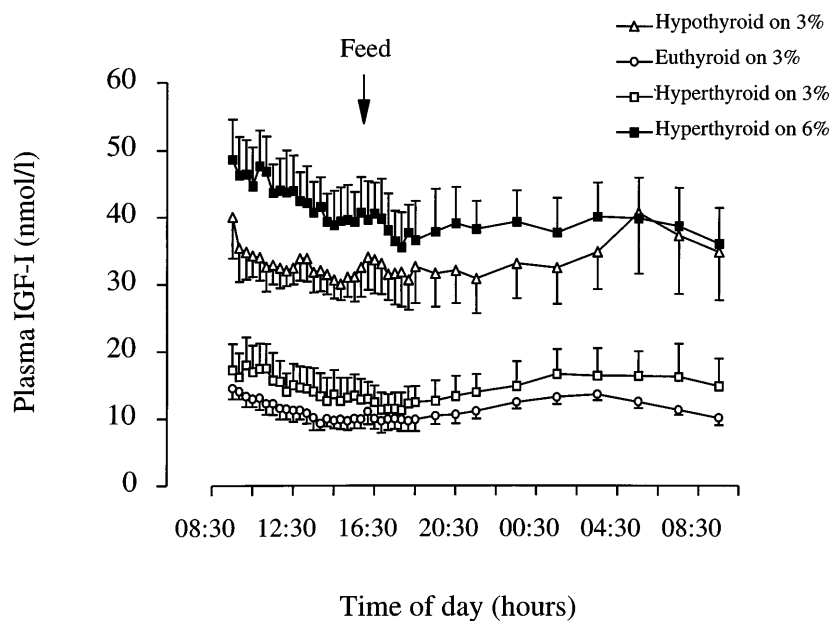


Figure 2 Plasma IGF-I concentrations in the 4 treatment groups over a 24-h period. Animals were 8 weeks of age, and were fed once per day at the indicated time. Food intake levels are shown as percentage of body weight for each group. Hyperthyroid animals received T₄ orally with food during the 4-week period of treatment, but did not receive their T₄ dose on the day of sampling. Each value is mean \pm s.e.m. for 5 animals.

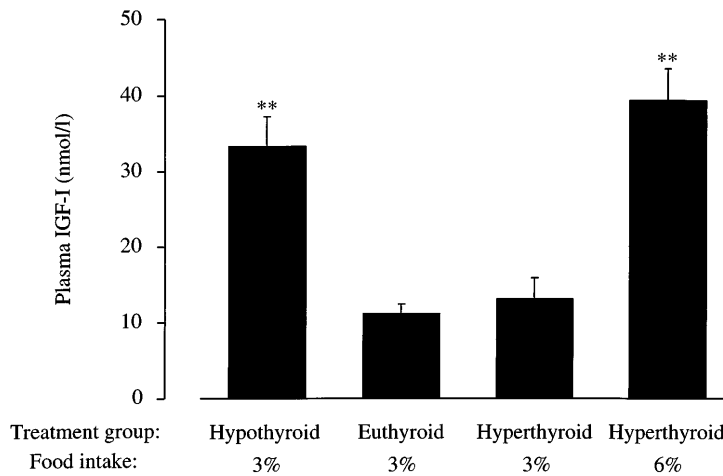


Figure 3 Mean \pm S.E.M. plasma IGF-I concentrations for 5 animals in each of the 4 treatment groups. For each animal, the IGF-I value was taken as the mean of 37 samples collected over a 24-h period. Food intakes are shown as percentage of body weight for each group. ** $P < 0.01$ compared with other groups.

taken over the sampling period were averaged for each animal, and used to establish a group mean (Fig. 5). A significant difference was found between different treatment groups ($P < 0.01$). Similar to the IGF-I results, hypothyroid animals on 3% food intake and the hyperthyroids on extra food had higher circulating insulin levels than the euthyroid controls and the hyperthyroids on 3% food intake ($P < 0.01$ for all comparisons).

Discussion

To our knowledge, these observations demonstrate for the first time that there is no direct correlation between thyroid status and plasma IGF-I concentration. Rather, the effects of THs on plasma IGF-I appear to be dependent on energy balance. The use of young growing pigs in this study has enabled manipulation of food intake to an extent that would be difficult in many

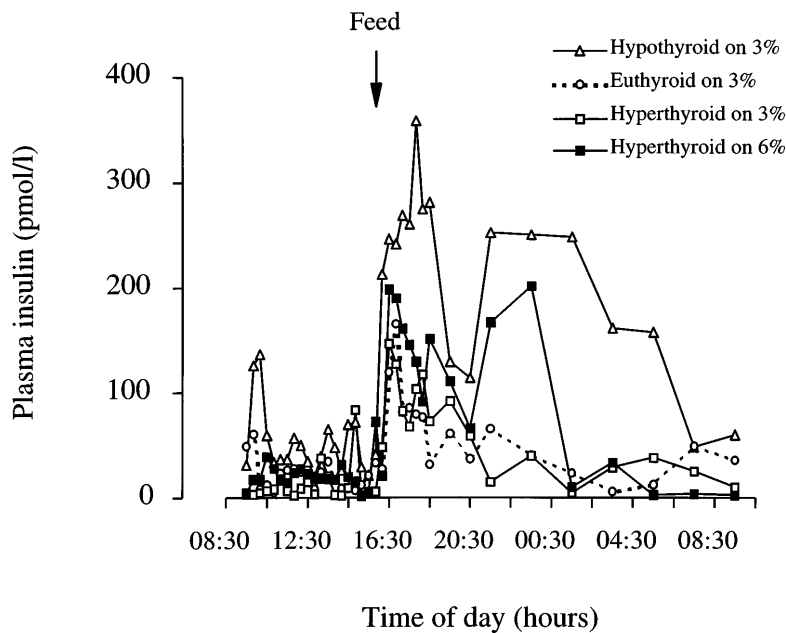


Figure 4 Plasma insulin concentrations in the 4 treatment groups over a 24-h period. Animals were 8 weeks of age, and were fed once per day at the indicated time. Food intake levels are shown as percentage of body weight for each group. Hyperthyroid animals received T_4 orally with food during the 4-week period of treatment, but did not receive their T_4 dose on the day of sampling. Each value is mean \pm S.E.M. for 5 animals.

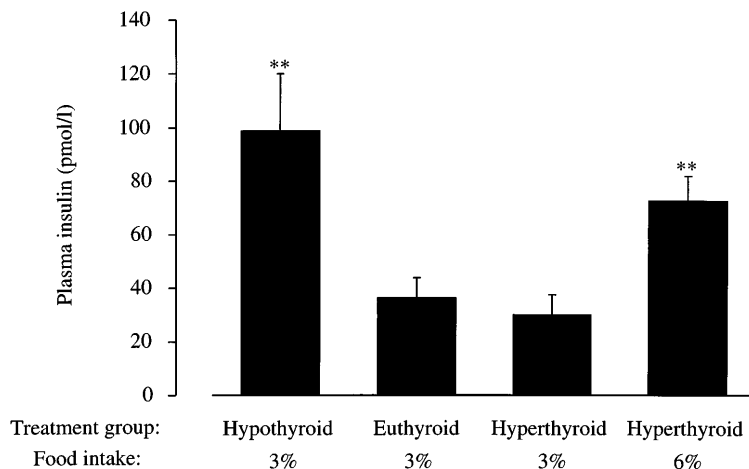


Figure 5 Mean \pm S.E.M. plasma insulin concentrations for 5 animals in each of the 4 treatment groups. For each animal, the insulin concentration was taken as the mean of 37 samples collected over a 24-h period. Food intakes are shown as percentage of body weight for each group. ** $P < 0.01$ compared with other groups.

other species, and at the same time has allowed assessment of the effects of the treatment conditions on body weight during a period of rapid growth. On a weight of food per body weight basis, the pig has a greater appetite than other domestic mammals. Pigs on 3% standard diet are well below their *ad libitum* food intake, which can exceed 5% body weight. In the current study, the hypothyroid animals and the hyperthyroids on extra food were close to their *ad libitum* food intake, and therefore had a greater energy intake relative to expenditure compared with the other two groups. This energy surplus may modify circulating levels of hormones that are involved in the regulation of growth and body composition in young animals. However, different underlying mechanisms were probably responsible for the increases in body weights in the hypothyroid group and in the hyperthyroid animals on extra food intake.

Because of the effects of THs on basal metabolic rate (BMR), voluntary food intake is generally decreased in hypothyroidism and increased in the hyperthyroid state (20). The reduction in BMR in hypothyroidism increases the ratio of energy intake to energy expenditure relative to euthyroid animals consuming the same amount of food. This ratio is also increased in hyperthyroid animals on twice the intake of food. The elevated insulin levels in hypothyroid animals may have at least two underlying causes. The increased adiposity that is generally associated with the hypothyroid state decreases the insulin sensitivity of peripheral tissues, thereby increasing insulin requirement, which is met by increased insulin secretion by the pancreas (29, 30). Alternatively, a direct decrease in insulin sensitivity in hypothyroidism, as has been suggested previously (31), may contribute to the elevated circulating insulin levels. By contrast with hypothyroid animals, the elevated plasma insulin concentration in the hyperthyroids is

related to an increased food intake compared with the euthyroid controls.

No previous data on the effects of THs and nutritional state on circulating GH in the pig are available. In animals on the same food intake, the current data demonstrate a decreased circulating GH level in both the hypothyroid state and in hyperthyroid animals on extra food intake. Since the peak GH heights are comparable among the treatment groups, the lower total GH peak area in hypothyroid animals compared with the euthyroid controls may be due to a lower baseline and an increased clearance of GH from the circulation, as may also be the case in hyperthyroid animals on extra food, and/or relatively fewer or narrower GH peaks. Although GH gene expression is generally modified by thyroid status, there appears to be some variation in the effect of THs on GH synthesis in different species. The plasma GH values from the hypothyroid animals in this study are in agreement with findings in the rat, in which hypothyroidism reduced the pituitary content of GH (32), and with those in hypothyroid humans, where GH secretion, both spontaneous and after stimulation by a variety of stimuli other than thyroid releasing hormone, is blunted (33). At least in pituitary cell lines, this reduction correlates well with a reduction in the number of TH receptors, suggesting a direct effect of TH on GH gene transcription (7). The converse, however, does not appear to occur in hyperthyroidism. Thus, whereas GH gene expression in rat somatotrophs is stimulated by THs (34), T_3 fails to increase basal or stimulated bovine pituitary GH secretion (35, 36), and has an inhibitory effect on the synthesis and secretion of GH in humans (37, 38). These previous findings in hyperthyroid humans agree only with the hyperthyroid animals in our study that were receiving extra food, suggesting a modifying role for nutrition on the influence of THs on plasma GH levels.

IGF-I synthesis and production are under the control of GH (1) and the nutritional state (15). The efficacy of GH in increasing circulating IGF-I levels, however, is dependent on energy availability. Previous studies have demonstrated that exogenous GH is incapable of increasing plasma IGF-I in the fasted state (17). This is in accord with our results that demonstrate no change in plasma IGF-I concentrations in the hyperthyroid state when food intake is not increased. Previous evidence suggests that this may be due to a reduction in the number of hepatic GH receptors (26).

The inverse relation between plasma GH levels and IGF-I in hypothyroids and in hyperthyroids on extra food may have different underlying mechanisms. Excessive weight gain is capable of blunting or abolishing GH output in response to a variety of stimuli independently of the thyroid status (39). The suppressed GH concentrations in these two groups may be partly due to feed-back inhibition by elevated circulating IGF-I concentrations. Increased circulating insulin in these two groups may also induce a reduction in GH secretion from the pituitary cells (19). The elevated IGF-I levels may be secondary to either an increased synthesis or a decreased clearance of the hormone. Previous studies in the rat have demonstrated hepatic GH binding capacity to be decreased in hypothyroidism and increased in hyperthyroidism (40). Moreover, obesity and increased insulin concentrations both decrease plasma IGF-binding protein-1 (41, 42), which aids delivery of IGF-I to tissues. This would favour a reduction in clearance and a decrease in biological activity of IGF-I in the hypothyroid state, as has been shown previously (5, 43). Young pigs on a high food intake also have increased GH receptor gene expression (26), which correlates well with the elevated hepatic IGF-I mRNA synthesis and plasma IGF-I concentrations (44). Therefore, in the presence of adequate energy intake in the hyperthyroid state, the increase in plasma IGF-I concentrations may be due to an increased synthesis of the hormone. Nevertheless, at least one previous study in adult humans has demonstrated a decrease in bioactivity of IGF-I in hyperthyroidism (5). Whether this also occurs in young growing individuals now needs to be determined.

In conclusion, we have demonstrated a close inverse correlation between the circulating concentrations of GH and IGF-I under the different experimental conditions studied. The plasma IGF-I concentrations correlated directly with the circulating insulin levels. However, there appears to be no direct linear relationship between thyroid status and GH and IGF-I concentrations in the young pig. Rather, the effects of THs on plasma IGF-I and GH appear to be modified by nutritional signals, which may involve plasma insulin concentrations. This is further evidence to support the importance of nutrition and energy balance in regulating circulating IGF-I levels. It remains to be determined how the interaction between thyroid status and nutrition may

modify the biological activity of IGF-I, as well as the number of IGF-I receptors and the postreceptor events in IGF-I bioactivity.

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