Effects of dietary energy supply on serum thyroxine, tri-iodothyronine and insulin concentrations in young horses

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ABSTRACT

The effects of meal ingestion on the circulating concentrations of the growth-regulating hormones thyroxine (T₄), tri-iodothyronine (T₃) and insulin were examined in weanling Thoroughbreds fed 70% (diet A), 100% (diet B) or 130% (diet C) of their energy and protein requirements. Peak insulin concentrations occurred 1, 2 and 3 h after the ingestion of diets C, B and A respectively. Increases in plasma glucose concentrations preceded the increases in serum insulin concentrations. Serum T₄ concentrations increased after the ingestion of diets A and B and decreased after diet C. In contrast, serum T₃ concentrations were unaffected by ingestion of diet A but increased after the ingestion of diets B and C. The increase was much greater and more rapid in the horses fed diet C. However, the decrease in T₄ concentration was five times greater than the increase in T₃ concentration. Accelerated insulin secretion after the ingestion of a meal high in energy (carbohydrate) content was therefore associated with decreased T₄ secretion and accelerated T₄ conversion to T₃. However, 6 months later serum T₄ and T₃ concentrations were unaffected by meal ingestion.


INTRODUCTION

Important interactions among dietary glucose contents, pancreatic insulin secretion rates and hepatic deiodination of thyroxine (T₄) to tri-iodothyronine (T₃) by the 5′-deiodinase enzyme have been demonstrated in man and rats. Fasting results in decreased serum insulin and T₃ concentrations (Vagenakis, Burger, Portnay et al. 1975; Kaplan & Utiger, 1978), decreased hepatic conversion of T₄ to T₃ (Vagenakis, Portnay, O’Brian et al. 1977; Suda, Pittman, Shimizu & Chambers, 1978) and decreased hepatic 5′-deiodinase activity (Cavaliere & Rapoport, 1977), while refeeding with a carbohydrate-rich diet is accompanied by increased serum T₃ and insulin concentrations, more rapid conversion of T₄ to T₃ (Gavin, McMahon & Moeller, 1980, 1981) and increased 5′-deiodinase activity (Gavin & Moeller, 1983). Refeeding with protein or fat does not elicit these effects (Gavin & Moeller, 1983). Cultured rat hepatocytes exhibit increased 5′-deiodinase activity when exposed to insulin but not when exposed to glucose (Sato & Robbins, 1981). Correlations between levels of carbohydrate refeeding and hepatic 5′-deiodinase activity have not been investigated. When weanling horses were fasted for 24 h and then fed a meal deficient in energy and protein, serum T₄ concentrations were increased above fasting levels for 4 h; feeding a meal containing twice as much energy and protein elicited T₄ concentrations which remained below fasting levels for 1.5 h (Glade, Gupta & Reimers, 1984). The decrease in serum T₄ concentrations were associated with rapid post-prandial increases in serum insulin concentrations.

Bone and joint deformities that accompany long-term overfeeding of young animals resemble those of hypothyroidism. The similarities suggest that transient post-prandial alterations in T₄ metabolism may be implicated in the development of joint disease in overfed growing animals (Glade et al. 1984). However, hepatic T₄ metabolism may adapt to chronic overfeeding. These hypotheses were tested in the present study by the long-term feeding of diets deficient, adequate or excessive in energy and protein to young growing horses.
**MATERIALS AND METHODS**

Twelve Thoroughbred weanling foals between 6 and 8 months old were assigned to groups by age and one of three corn and pelleted hay–concentrate diets was fed to each group. The diets were formulated so that they provided 70% (group A), 100% (group B) or 130% (group C) of National Research Council (1978) recommendations for joules of digestible energy and g protein (Table 1). All three diets provided 100% calcium and phosphorus requirements. The daily ration was divided into two meals per day. The animals were housed in 4 m square box stalls. Tap water, an iodized salt block and an individual exercise lot were available to each horse ad libitum.

**Table 1. Composition of the diets fed daily to young horses (per kg body wt)**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>A (70%)</th>
<th>B (100%)</th>
<th>C (130%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestible energy (J)</td>
<td>0.187</td>
<td>0.267</td>
<td>0.347</td>
</tr>
<tr>
<td>Crude protein (g)</td>
<td>2.27</td>
<td>3.24</td>
<td>4.22</td>
</tr>
<tr>
<td>Calcium (g)</td>
<td>0.14</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Phosphorus (g)</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Starch (g)†</td>
<td>2.44</td>
<td>3.48</td>
<td>4.52</td>
</tr>
</tbody>
</table>

* Percentages of digestible energy and crude protein intakes recommended by the National Research Council (1978).
† Assuming corn contains 50% starch (Hintz, Hogue, Walker et al. 1971).

After being fed the experimental diets for 1 month the horses were fasted for 24 h (experiment 1). Jugular venous blood samples were drawn and the horses were then fed. Additional blood samples were drawn immediately after all feed had been consumed and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5 and 6 h in order to monitor changes in glucose and hormone concentrations after feeding. Sampling times chosen were based on the results of a previous pilot study (Glade et al. 1984). Serum and plasma fractions were separated and stored at −4 °C.

After being fed the experimental diets for another 6 months the horses were again fasted and fed (experiment 2). Sequential blood samples were drawn before feeding, after half of the feed had been consumed, at meal completion and at 1, 2, 3 and 4 h after feeding.

Plasma glucose concentrations were determined by colorimetric procedures (Technicon Autoanalyzer Method N 16 B, Technicon Corp., Tarrytown, New York, U.S.A.). Serum concentrations of T₃ and T₄ were determined by radioimmunoassays (Antibodies, Inc., Davis, California, U.S.A.) validated for equine T₃ and T₄ (Reimers, Cowan, Davidson & Colby, 1981). The intra- and interassay coefficients of variation for T₄ were 6-7 and 9-2% respectively, and for T₃ they were 10-2 and 12-2%. Serum insulin concentrations were measured by a radioimmunoassay technique (MicroMedic Systems, Horsham, Pennsylvania, U.S.A.) which had been validated for equine insulin (Reimers, Cowan, McCann & Ross, 1982). The intra- and interassay coefficients of variation were 10-7 and 17-8% respectively.

The results from each experiment were analysed by analyses of variance for repeated measurements (split-plot) using the Statistical Analysis System computer package (Barr, Goodnight, Sall et al. 1979). When the F ratio for sampling times was significant (P<0.05), comparisons among individual mean concentrations of a given variable at different sampling times were made by the conservative application of Tukey's Honestly Significant Difference test (Steel & Torrie, 1960). Comparisons among diet means at given sampling times were made by the same method when the F ratio for diet x sampling-time interactions was significant (P<0.05).

**RESULTS**

**Experiment 1: Post-feeding changes in serum hormone concentrations in foals 6–8 months old**

Concentrations of T₃, T₄, insulin and glucose were not different among these dietary regimens after 24 h of fasting (Fig. 1). Overall main diet effects on post-prandial concentrations were significant (P<0.01) only in the case of T₃. However, the effects of sampling time and of diet by sampling-time interactions were significant for each variable (P<0.05), indicating that the responses to meal ingestion depended on both the size of the meal and the time after meal consumption.

Glucose concentrations began rising in all animals during their meals (Fig. 1). At meal completion, glucose concentrations were 25, 16 and 53% higher than were fasting concentrations in groups A, B and C respectively. The increase was significant in group C (P<0.05). Within the next 0.25 h, there were additional 30 and 25% increases in groups A and B (P<0.05), while glucose concentrations in group C had begun to plateau. Glucose concentrations began to decrease at 2 h in group C and at 3 h in groups A and B (P<0.05), although in groups A and B they were not significantly different from peak concentrations until 4 h.

Average insulin concentrations were doubled at meal completion compared to prefeeding concentrations in group A and were tripled in group B (Fig. 1), but these increases were not statistically significant. The horses in group C exhibited a significant (P<0.05) fourfold increase in mean insulin concentration at this
time, coincident with the significant increase in glucose concentration. Insulin concentrations continued to increase in all groups, reaching similar maximum levels at 3 h in group A, 2 h in group B and 1 h in group C. Time to attain peak insulin concentration was inversely proportional to dietary intake (P<0.05). Subsequent decreases occurred in a similar sequence, beginning at 4 h in groups A and B and at 2 h in group C.

At meal completion, mean T₄ concentrations were 70% greater than fasting concentrations in group A (P<0.05), but were increased only 20% in group B and were decreased 10% in group C (Fig. 1). Thereafter, T₄ concentrations in group A decreased for 2 h (P<0.05), then stabilized at a level 33% higher than the 24-h fasting level (P<0.05). In group B, T₄ concentrations reached a maximum at 0.25 h (P<0.05), began decreasing at 1.5 h and returned to fasting levels at 4 h. At 0.5 h after meal completion, T₄ concentrations in group C reached a minimum, after which they increased steadily through 6 h to levels slightly higher than those observed at the end of the fast (P>0.05).

Serum T₃ concentrations were not affected by feeding in group A (Fig. 1). In group B, T₃ concentrations began increasing after meal completion, reached a maximum at 2 h and returned to fasting levels at 6 h. Serum T₃ concentrations increased 140% during meal ingestion in group C (P<0.01). This level was maintained for 0.5 h, after which serum T₃ concentrations steadily declined.

**Experiment 2: Post-feeding changes in serum hormone concentrations in yearlings 12–14 months old**

Main effects of diet were significant (P<0.01) for insulin and T₃ but not for T₄ or glucose. The effects of sampling time and of diet by sampling-time interactions were significant (P<0.05) for insulin, glucose and T₃ but not for T₄.

Accordingly, T₄ concentrations were not different among diet groups at any time, nor did they vary with time after the animals were fed (Fig. 2). However, they were an average of 46% higher after fasting in experiment 2 than in experiment 1.

Serum T₃ concentrations exhibited brief increases in each group at different times after meal completion (Fig. 2). However, these changes did not follow any pattern.

Average glucose concentrations were not different at prefeeding and increased in all horses during the meal (Fig. 2). By meal completion glucose concentrations were significantly raised over fasting concentrations in all animals (P<0.05), with the increase occurring more rapidly in group C. Glucose concentrations reached their maximums in all groups at 2 h and then decreased, the decrease beginning after 2 h in group C and after 3 h in groups A and B.

Changes in insulin concentrations followed patterns similar to those observed in experiment 1 (Fig. 2).
DISCUSSION

The results of experiment 1 confirm that the changes in post-prandial T\(_4\) concentrations in weanling horses are dependent on the amount of dietary energy (carbohydrate) and protein consumed, as previously reported (Glade et al. 1984). Meals containing moderate amounts of carbohydrates (diets A and B) were associated with increases in serum T\(_4\) concentrations during the first several hours after meal completion. These observations are consistent with reports that T\(_4\) secretion rates and serum T\(_4\) concentrations are increased after the ingestion of meals containing carbohydrate by pigs (Ingram & Kaciuba-Uscilko, 1977; Ingram & Evans, 1980; Dauncey, Ingram, Macari & Ramsden, 1982), man (Spaulding, Chopra, Sherwin & Lyall, 1976; Danforth, Horton, O'Connell et al. 1979) and cattle (Blum, Gingins, Schnyder et al. 1979a; Blum, Thomson & Bickel, 1979b). In addition, thyrotrphin (TSH) secretion has been reported to increase after feeding in rats (Hugues, Burger, Grousalie et al. 1983) and man (Hugues, Burger, Pekary & Hershman, 1984).

Serum T\(_3\) concentrations were unchanged after the low-carbohydrate meal. Reduced caloric feeding has failed to alter serum T\(_3\) concentrations in cattle (Blum et al. 1979b). The mild increases in serum T\(_3\) concentrations after the adequate meal were similar to those reported to occur in moderately fed man (Danforth et al. 1979; Hugues et al. 1984).

In contrast, a meal presumably exceeding requirements by 30% resulted in decreasing T\(_4\) and increasing T\(_3\) concentrations during and immediately after feeding, suggesting increased conversion of T\(_4\) to T\(_3\). Increases in T\(_3\) concentrations after high-carbohydrate meals have been associated with accelerated peripheral conversion of T\(_4\) to T\(_3\) in rats (Gavin & Moeller, 1983), pigs (Ingram & Evans, 1980; Dauncey et al. 1982), chickens (Harvey & Klandorf, 1983) and man (Danforth et al. 1979). Post-prandial T\(_4\) concentrations decreased and T\(_3\) concentrations increased as the apparent rate of insulin secretion increased in this study. These observations are consistent with reports that increased hepatic T\(_4\)-5'-deiodinase activity is associated with increased insulin concentrations in carbohydrate-fed rats (Gavin & Moeller, 1983) and in cultured rat hepatocytes (Gavin, Bissell, Hammond & Cavalieri, 1978; Sato & Robbins, 1981).

It is possible that the increased serum T\(_3\) concentrations resulted from decreased conversion of T\(_4\) to its inactive metabolite 3,3',5'-triiodothyronine (rT\(_3\)), while the rate of T\(_4\) conversion to T\(_3\) (3,5,3'-triiodothyronine) did not change. However, changes in T\(_3\) and rT\(_3\) production rates have been shown to behave in a reciprocal fashion, increases in one being linked to

\[ \text{FIGURE 2. Concentrations of glucose in the plasma and of tri-iodothyronine (T}_3\text{), thyroxine (T}_4\text{) and insulin in the serum of fasted yearling horses fed meals providing 70\% (group A), 100\% (group B) or 130\% (group C) of energy and protein requirements (experiment 2). P indicates pre-feeding sample. M indicates mid-meal sample. Values are means ± S.E.M. (n = 4). Comparisons among means are given in the text.} \]
decreases in the other, in man (Danforth et al. 1979; Hugues et al. 1984) and cattle (Blum et al. 1979b).

The decreases in T₄ concentrations observed in experiment 1 (4.0 nmol/l) were much larger than the increases in T₃ concentrations (0.95 nmol/l), suggesting that a meal high in carbohydrate content may have inhibited T₄ secretion as well as accelerating T₃ production. Rojmark & Nygren (1983) reported that insulin infusion inhibited pituitary secretion of TSH following a thyrotrophin-releasing hormone (TRH) challenge. In this study post-prandial insulin concentrations reached their maximum levels most rapidly in group C, during the time when T₄ concentrations were decreasing. Insulin-induced pituitary refractoriness to TRH may have contributed to the decline in T₄ concentrations.

The failure to observe changes in post-prandial concentrations of thyroid hormones in experiment 2 may indicate age-related changes in the sensitivity of hepatic enzymes or the pituitary gland to insulin. Such an effect of ageing may have been reported to occur in senescent rats (Gambert, 1982).

Thyroid hormones are required for the biochemical maturation of cartilage (Hoskins & Asling, 1977; van Buul & van den Brande, 1978; Holder, Wallis, Biggs & Preece, 1980; Reddi & Sullivan, 1980). Whether the changes in serum T₃ and T₄ observed in experiment 1 were expressed through effects on the maturation of cartilage remains unknown. Nonetheless, diminished thyroid control of cartilage development could explain the paradox in which excessive nutrition is associated with the production of an excessive amount of developmentally immature cartilage.

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REFERENCES


