Dietary Carbohydrate and Fat Do Not Alter the Thyroid Response to Protein Deficiency in Chicks (44116)

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Abstract. **Consumption of low-protein diets consistently causes elevations in circulating levels of triiodothyronine (T_3) in several species of animals. In chicks this is often accompanied by lower levels of circulating thyroxine (T_4). Since low-protein diets are usually formulated by replacing the deleted protein with carbohydrate, the question arises as to whether the changes in thyroid hormones are a result of the lower protein or higher amounts of carbohydrate in such diets. Male broiler chicks, 13–26 days of age, were fed experimental diets that contained either an adequate level of protein (24%) or levels that were slightly (17%) or moderately (10%) deficient in protein. The deleted protein was replaced, isocalorically, with either glucose, soybean oil, or hydrogenated coconut fat. Though the level of protein and source of energy differed among diets, all diets contained identical amounts of all nutrients and energy, and were of similar weight densities. Circulating levels of thyroid hormones were measured from blood samples taken at the end of the study. Plasma T_3 was elevated to a similar degree in all protein-deficient animals compared with control. Plasma T_4 decreased in all protein-deficient chicks and was lowest with 10% dietary protein. Changes in circulating levels of thyroid hormones occurred independently of the source of dietary energy. Therefore, it is concluded that alterations in circulating levels of thyroid hormones that occur in chicks fed low-protein diets are a specific effect of the protein deficit and are not a related to the amounts of carbohydrate or fat present in the diet.** [P.S.E.B.M. 1997, Vol 215]

Research has shown repeatedly that consumption of protein-deficient diets by growing chicks causes elevations in circulating levels of blood triiodothyronine (T_3), accompanied frequently by decreases in blood levels of thyroxine (T_4) (1–3). This may (3) or may not (1, 2) be accompanied by changes in efficiency of energy use. When protein-deficient diets are formulated in such studies, it is most common to replace the deleted protein with equal quantities of carbohydrate, usually glucose. The possibility arises from this that it is actually the higher carbohydrate content of the deficient diets rather than the lower protein content that causes alterations in circulating levels of thyroid hormones.

Similar increases in circulating levels of T_3 occur when rats are fed low-protein, high-carbohydrate diets, although circulating levels of T_4 are within the normal range or slightly elevated (4–6). It has been suggested that a high intake of carbohydrate itself is responsible for elevations in levels of circulating T_3 in humans (7) and rats (4), although Tulp *et al.* (8) suggested that changes in thyroid hormone levels, at least in protein-deficient rats, occur independently of changes in dietary carbohydrate and fat.

The purpose of the present study was to determine if changes in circulating levels of thyroid hormones in protein-deficient chicks occur independently of changes in the carbohydrate and fat fractions of the diet. Also, the effects of two fats differing widely in composition, one polyunsaturated and the other highly saturated, were compared.

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Table I. Composition of the Constant-Ingredients Premix

Ingredient	Percentage in diet
Glucose (Cerelose)[a]	26.657
Soybean oil	4.000
Cellulose (Solka-Floc)[b]	3.000
Mineral mixture[c]	5.630
Vitamin mixture[d]	0.500
Choline chloride (70%)	0.200
Ethoxyquin[e]	0.013
Total	40.000 of each diet

[a] CPC International, Inc. (Englewood Cliffs, NJ).
[b] Brown Co. (Berlin, NH).
[c] Supplies in g/kg of complete diet: $CaHPO_4 \cdot 2H_2O$, 20.7; $CaCO_3$, 14.8; KH_2PO_4, 10.0; KCl, 1.0; NaCl, 6.0; $MnSO_4 \cdot H_2O$, 0.35; $FeSO_4 \cdot 7H_2O$, 0.5; $MgSO_4$, 3.0; KIO_3, 0.002; $CuSO_4 \cdot 5H_2O$, 0.03; $ZnCO_3$, 0.15; $CoCl_2 \cdot 6H_2O$, 0.0017; $Na_2MoO_4 \cdot H_2O$, 0.0083; Na_2SeO_3, 0.0005.
[d] Supplies in mg/kg of complete diet: riboflavin, 15; *d*-pantothenate Ca-salt, 50; niacin, 100; pyridoxine HCl, 15; thiamin HCl, 15; folacin, 6; biotin, 0.5; vitamin B_{12}, 0.05; menadione Na bisulfite (63%–75%), 5; vitamin E mix (500 IU/g), 100; vitamin A mix (650,000 IU/g), 15; vitamin D_3 mix (200,000 IU/g), 24; glucose, 4654.5.
[e] 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline; Monsanto Chemical Co. (St. Louis, MO).

Materials and Methods

Diets. A nutritionally complete, semipurified, control diet, constituted primarily of purified soybean protein and glucose, was used throughout the study. This is a slight modification of the diet described by Scott *et al.* (9). It was composed of a constant-ingredients premix, which was used at the same level (40%) in all experimental diets (Table I), and of variable components which consisted of protein, amino acids, glucose, and fat (Table II). Experimental diets (Table II) were formulated to contain adequate protein (24%) or deficient levels at either 17% or 10% of the diet by removing part of the soybean protein and replacing it with either glucose or one of two types of fat. The levels of added methionine and glycine were adjusted such that the amino acid:protein ratios in all diets were constant. The deleted soy protein in the deficient diets was replaced isocalorically with either glucose, soybean oil, or hydrogenated coconut fat using the following metabolizable energy values (in kcal/g): isolated soy protein, 3.56; glucose, 3.34; soybean oil, 8.95; and hydrogenated coconut fat, 7.23. The diets were also maintained at uniform weight:volume density within each protein level by adding a 75/25 mixture of cellulose and sand (sifted through a 841-micron screen) to counterbalance the higher caloric density of the fats compared with glucose.

Animals. Four hundred day-old male broiler chicks were obtained from a commercial hatchery (Arbor Acres Farms, Inc., Glastonbury, CT). They were all maintained on the 24% protein control diet for 12 days, at which time they were distributed, as evenly as possible based on body weight, into 21 pens of 10 chicks each (3 pens in each of 7 treatments) using chicks from the mid-range of body weights. They were fed the experimental diets, free-choice, from 13 to 26 days of age, at which time body weights and blood samples were taken. Chicks were housed in Petersime, electrically-heated battery brooders with raised wire floors. They were allowed free access to water and given a 16:8-hr light:dark lighting cycle daily.

Blood Analysis. Blood was drawn between 1000 and 1300 hr from each chick by side heart puncture and placed into heparinized tubes. The tubes were centrifuged at 1800*g* for 15 min, and the plasma was removed and frozen for later analysis. Plasma total T_3 and T_4 levels were analyzed by radioimmunoassay as described previously (2).

Statistics. Data were analyzed by analysis of variance, and means were compared with Duncan's multiple range test (10). $P \leq 0.05$ was considered significant.

Results

Weight Gain. Chicks consuming the protein-deficient diets had significantly smaller weight gains (Table III). Weight gains of all chicks fed 10% protein were significantly less than those fed 17% protein. Within the

Table II. Composition of Experimental Diets

Ingredient	24% protein control	17% protein	17% protein + soy oil	10% protein + coconut fat	10% protein	10% protein + soy soil	10% protein + coconut fat
Premix	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Soy protein[a]	26.6	18.8	18.8	18.8	11.1	11.1	11.1
DL-methionine	0.6	0.42	0.42	0.42	0.25	0.25	0.25
Glycine	0.4	0.28	0.28	0.28	0.17	0.17	0.17
Glucose	32.4	40.5	32.4	32.4	48.48	32.4	32.4
Soybean oil	—	—	3.02	—	—	6.0	—
Coconut fat[b]	—	—	—	3.75	—	—	7.45
Cellulose/sand mix[c]	—	—	5.08	4.35	—	10.08	8.63

Note. All values are percentages. All diets within a protein level (IE, 17% or 10%) were formulated to be isocaloric and of the same density.
[a] Isolated soybean protein Archer Daniels Midland Co. (Decatur, IL).
[b] Procter and Gamble (Cincinnati, OH).
[c] This mixture mimics the density of glucose.

Table III. Effect of Dietary Protein, Fat, and Carbohydrate Contents on Growth, Feed Intake, Feed-to-Gain Ratio, and Plasma Levels of Triiodothyronine (T_3) and Thyroxine (T_4) in Male Broiler Chicks 12–26 Days of Age

Dietary protein level	Ingredient replacing protein[a]	Weight gain (g)	Feed intake (g)	ME intake[b] (kcal)	Feed:gain (g:g)	Plasma T_3 (ng/ml)	Plasma T_4 (μg/dl)
24% control	—	635 ± 7^{c} (28)	859 ± 13^{d} (3)	3161	1.35 ± .02^{d} (3)	1.82 ± .11^{d} (18)	1.81 ± .08^{c} (15)
17%	glucose	602 ± 9^{e} (30)	968 ± 3^{c,e} (3)	3543	1.61 ± .01^{e} (3)	2.45 ± .12^{c,e} (17)	1.59 ± .09^{e} (13)
17%	soybean oil	588 ± 13^{e} (30)	929 ± 56^{d,e} (3)	3400	1.58 ± .00^{e} (3)	2.37 ± .10^{c,e} (18)	1.53 ± .04^{e} (15)
17%	coconut fat	616 ± 8^{e} (30)	979 ± 35^{c,e} (3)	3583	1.59 ± .05^{e} (3)	2.17 ± .10^{e} (20)	1.61 ± .06^{e} (15)
10%	glucose	359 ± 10^{f} (29)	857 ± 18^{d} (3)	3119	2.40 ± .07^{c} (3)	2.40 ± .14^{c,e} (16)	0.88 ± .08^{d} (15)
10%	soybean oil	455 ± 12^{d} (29)	1040 ± 8^{c} (3)	3786	2.29 ± .04^{c} (3)	2.53 ± .12^{c} (18)	1.06 ± .06^{d} (14)
10%	coconut fat	434 ± 10^{d} (30)	990 ± 3^{c,e} (3)	3604	2.28 ± .02^{c} (3)	2.45 ± .08^{c,e} (20)	0.99 ± .06^{d} (15)

Note. Numbers in parentheses under mean values represent the number of observations. Means (±SEM) within a column with no common superscripts ($^{c\text{-}f}$) are significantly different ($P < 0.05$).
[a] Substitutions were made on an isocaloric basis.
[b] Metabolizable energy (ME) intake is calculated as feed intake (g/chick) × ME of diet (kcal/g).

treatments fed 17% protein, weight gains were similar regardless of the source of nonprotein energy. However, with 10% dietary protein the substitution of protein with either type of fat permitted significantly greater weight gains than when protein was substituted with glucose, but there was no significant effect due to type of fat.

Feed and Energy Intake. With a deficiency of 17% protein, both feed and energy intakes of all three treatments were significantly greater than with chicks fed the 24% protein control diet *ad libitum*, and did not differ from each other (Table III). On the other hand, chicks fed 10% protein where glucose had been substituted for protein had feed and energy intakes similar to the *ad libitum*–fed controls, but, when either type of fat was used as the substitute for the protein, feed intake increased significantly.

Feed:Gain. The net effect of changes in weight gain and feed intake was that the feed:gain ratio increased significantly as the level of dietary protein decreased and was significantly larger with 10% protein than with 17% protein.

Thyroid Hormones. Plasma T_3 levels were elevated in all protein-deficient chicks compared with the *ad libitum*–fed control group (Table III). Within the treatments receiving either 17% or 10% protein, plasma T_3 levels were not significantly different. There was a small but significant difference between the group fed 17% protein with coconut fat and the group fed 10% protein with soybean oil. In contrast, plasma T_4 levels were lower in all protein-deficient chicks compared with the *ad libitum*–fed control group. There were no significant differences among treatments receiving either 17% or 10% protein, but all treatments receiving 10% protein had significantly lower plasma T_4 levels than those fed 17% protein (Table III).

Discussion

This study clearly shows that elevations in plasma T_3 repeatedly observed in protein-deficient chicks, as well as decreases in plasma T_4, occur independently of the carbohydrate or fat contents of the diet. This demonstrates that the elevation in plasma T_3 observed in previous studies with protein-deficient chicks is not a consequence of the high carbohydrate content of deficient diets due to substitution of the protein source with carbohydrate; rather, it occurs even when the carbohydrate content of the diet is held constant by substituting dietary protein with fat. Therefore, alterations in circulating levels of thyroid hormones in such studies are truly a consequence of the protein deficit.

Whether or not a similar situation holds with other species is unclear because of limited data. Although it has been suggested that carbohydrate *per se* is responsible for the high levels of circulating T_3 in protein-malnourished humans (7) and rats (4), one limited report in abstract form suggests that serum T_3 increases in protein-deficient rats regardless of whether or not dietary protein is substituted with carbohydrate or fat (8), but statistical support for the data is not given.

That a protein deficiency itself may be responsible for the elevated plasma T_3 is strengthened by observations that deficiencies of certain amino acids, such as tryptophan (11) and arginine, lysine, isoleucine, or methionine (12) in chick diets, or valine in rat diets (4), also cause elevations in blood T_3.

The metabolic alterations that lead to changes in thyroid hormone levels in low-protein nutritional states are not well understood. Mechanisms that have been studied include increases in thyroid gland secretions, alterations in binding proteins, changes in receptor binding or affinity, increases in deiodinase enzyme activity, and decreases in metabolic clearance.

Studies of thyroid tissue morphology suggest that thyroidal secretions increase in protein-deficient rats (5). In chicks results are conflicting. March *et al.* (13) found lower thyroidal uptake of I^{131} in protein-deficient chicks and concluded that thyroid activity is decreased. In contrast, Keagy *et al.* (2) found no alterations in thyroid weight in protein-deficient chicks, suggesting no change in glandular function.

Smallridge *et al.* (14) suggested that elevations in plasma T_3 levels in protein-malnourished rats are due to an increase in protein bound T_3. Although increases in protein binding might not be expected in a protein deficiency, serum albumin levels are less affected by the deficiency than serum total protein levels (14), and in some reports T_3 was found to bind to albumin alone (15). Recently, Rouaze-Romet *et al.* (16) reported that thyroxine-binding globulin increases in rats fed low-protein diets. Serum T_4-binding activities were increased, although a decrease in transthyretin, the second specific T_4 carrier in rat serum, occurred concomitantly. Therefore, it is possible that alterations in affinity or absolute concentration of T_3 to serum protein or the presence of a new protein that preferentially binds T_3 may occur in protein-deficient animals.

Changes in thyroid receptors may also account for elevated T_3 levels. However, no changes in maximum binding capacity or binding affinity of hepatic receptors for T_3 were found in protein-deficient rats (14).

Increased deiodinase activity in protein-deficient animals would also result in increased production of T_3 from T_4. The decrease in plasma T_4 that accompanied the rise in plasma T_3 in the present study would suggest that this is the case. However, no changes have been found in hepatic or renal 5′-monodeiodinase activities in either protein-deficient rats (14, 17) or chicks (18, 19). Furthermore, the greater decrease in plasma T_4 relative to the smaller increase observed in plasma T_3 and the fact that T_4 decreased further at each level of protein deficiency whereas T_3 levels did not rise in such a graded fashion suggest that T_4 is being metabolized by other routes.

It has been suggested that decreased metabolic clearance of T_3 could explain the elevated T_3 in protein-deficient animals (20), but this seems unlikely since total T_3 is generally cleared more quickly than T_4 because it is not as tightly bound to plasma protein.

In the present study, chicks fed the diet most deficient in protein (10%) grew faster when either saturated or unsaturated fat rather than glucose was substituted for the protein source. This effect did not occur with the milder protein deficiency of 17%. Several studies, mainly with rats, have shown that addition of fats to diets adequate or limited in protein has a sparing effect on protein metabolism that allows the animal to make more efficient use of the limited dietary protein available for tissue synthesis (21–24). However, most of these studies show that carbohydrates, as well, has such a sparing effect on protein catabolism, although the effects may not be identical (25). Furthermore, in chicks it has been reported that medium-chain triglycerides, similar to the coconut fat used in the present study, have a more beneficial effect on protein utilization than do long-chain triglycerides such as found in soybean oil (26). Therefore, it is doubtful that the faster growth rate observed with both coconut fat and soybean oil in the 10% protein diet is the result of a protein-sparing effect.

This faster growth might also be a result of greater energy available from fat compared with glucose or, conversely, the lower heat produced when fat is substituted for glucose in chick diets (27). However, Carew *et al.* (28) have shown that this beneficial effect of dietary fat on efficiency of energy metabolism disappears when coconut fat is used in place of polyunsaturated fats such as soybean oil. Thus, the reason that fat substitution for glucose in severely protein deprived chicks stimulates growth and appetite is not understood and deserves further study.

In conclusion, the results of the present study demonstrate that changes in levels of circulating thyroid hormones in protein-deprived chicks is a specific result of the protein deficiency and is not a consequence of the high carbohydrate levels often found in low-protein diets.

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