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Chromium Supplementation of Turkeys: Effects on Tissue Chromium

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Four groups of 33-week-old turkey hens were fed either the basal diet for laying hens or the basal diet supplemented with 25, 100, or 200 μg of chromium as chromium chloride/g of diet. Liver Cr concentrations of the turkeys sacrificed after 5 weeks were 1.9, 36, 168, and 326 ng/g (wet weight). Similar trends, but higher chromium values, were observed for kidney samples. The chromium concentrations of the dark and white meat, eggs, and other edible tissues were not increased sufficiently for these tissues to serve as sources for Cr-enriched foods. Therefore, turkey liver is suitable for Cr enrichment studies while the eggs, dark and white meat, and other edible parts do not appear to be enriched sufficiently for these tissues to be used as sources of experimental high-Cr foods.

The absorption of dietary chromium (Cr) in humans eating freely chosen diets is inversely related to Cr content of the diet (Anderson and Kozlovsky, 1985). As a result of this inverse relationship of Cr absorption and dietary intake, the urinary excretion of Cr at intakes below 40 μg/day is relatively constant. At daily Cr intakes above 40 μg, absorption appears constant at approximately 0.4% and urinary losses increase with increasing intake (Anderson et al., 1983). However, daily Cr intakes in excess of 50 μg, do not usually occur unless subjects are taking supplements containing Cr.

To identify Cr-rich foods both suitable for human consumption and providing potential Cr intakes in excess of 50 μg/day, turkeys were supplemented with varying amounts of Cr. Turkeys were chosen for their size and suitability of the various edible parts as sources of food for human consumption. Turkeys could also be labeled with stable isotopes of Cr, and use of these labeled parts as enriched food sources will greatly facilitate Cr absorption studies in humans.

MATERIALS AND METHODS

Large white breeder hens, 33 weeks of age, were housed individually in suspended galvanized cages. The temperature of the room was maintained at 21 ± 1 °C, humidity 70 ± 5, with 14-h light and 10-h dark cycle. Turkeys were fed ad libitum a corn–soybean meal based diet containing 17% crude protein (Rosebrough and Steele, 1985) (Cr concentration 506 ± 59 ng/g) and this diet supplemented with 25, 100, and 200 μg of Cr as chromic chloride/g of diet. Two turkeys in each group were sacrificed after 1 and 5 weeks. Turkeys were sacrificed by cervical dislocation and the turkey parts removed with use of chromium-free plastic gloves; parts were rinsed with

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Table I. Analysis of Time and Dietary Chromium Effects on Wet Weight Chromium Concentrations of Turkey Tissues[d]

			Cr,[b] μg/g						
tissue	week	water, %	basal	basal + 25 Cr[a]	basal + 100 Cr[a]	basal + 200 Cr[a]	pooled SEM	slope linear	SE[c]
breast	1	72	1.1	1.2	1.9	1.3	0.30	0.014	0.0051
	5	72	0.8	1.1	2.8	3.5	0.30	0.027	0.0051
leg	1	73	5.4	8.0	5.1	6.5	0.98	0.007	0.0050
	5	73	0.8	1.0	2.0	3.7	0.98	0.007	0.0050
gizzard	1	75	2.3	4.3	11	19	3.08	0.070	0.0117
	5	75	1.0	1.9	8.0	12	3.08	0.070	0.0117
heart	1	77	2.2	2.4	4.4	7.0	0.85	0.025	0.0055
	5	77	0.9	1.8	8.0	12	0.85	0.056	0.0055
spleen	1	75	4.3	6	16	32	8.8	0.140	0.0510
	5	75	3.7	13	50	67	8.8	0.330	0.0510
pancreas	1	66	12	16	15	16	4.2	0.013	0.0248
	5	66	6.4	8	22	25	4.2	0.100	0.0248
lungs	1	75	16	21	30	39	7.8	0.100	0.0450
	5	75	12	12	41	59	7.8	0.25	0.0450
liver	1	66	7.0	15	53	72	9.4	0.33	0.0550
	5	66	1.9	36	168	326	9.4	2.6	0.0550
kidney	1	76	6.0	32	131	179	21	0.88	0.1560
	5	76	3.3	88	224	541	21	2.60	0.1560

[a] Micrograms of Cr added/gram of diet as chromium chloride. [b] Cr concentration based on wet weight. Each value is the mean of triplicate determinations of a sample from the respective tissues from two turkeys. [c] Standard error of linear slope. [d] Large white breeder laying hens, 33 weeks at onset of study (hens were just starting egg production).

Table II. Mean Squares of Dependent Variables[a]

sources of variation	DF	breast	leg	gizzard	heart	kidney	liver	pancreas	spleen	lungs
Cr linear	1	5.34*	4.27	470.67*	157.4*	296487*	93856*	312.18*	5369.0*	2957.0*
Cr quadratic	1	1.11*	0.47	4.49	1.53	37	166	35.30	95.8	37.4
week	1	1.79*	77.00*	47.61	9.77*	64592*	37037*	0.86	1402.5*	42.3
Cr linear × week	1	3.84*	5.84	14.99	22.52*	72449*	41007*	182.04*	814.8*	586.5*
Cr quadratic × week	1	0.01	0.05	0.03	2.48	4534	49	21.93	170.6	0.7
error	10	0.16	2.53	15.33	1.34	954	152	30.09	126.8	111.5

[a] Asterisk denotes a significant effect at $P < 0.05$.

deionized water and frozen in plastic bags until analyses. In a separate study, eggs were collected from 12 turkeys for four consecutive weeks following Cr supplementation at 25 and 100 μg of Cr/g of diet.

Immediately before use, tissue samples were thawed, cut into small pieces with a titanium knife, and weighed into borosilicate glass tubes in triplicate. Samples were freeze-dried and ashed 16 h in a muffle furnace, stepwise to 480 °C. Fifty microliters of deionized water, 50 μL of Ultrex nitric acid, and 100 μL of 50% hydrogen peroxide were added to ashed samples. Samples were dried in a heating block, dissolved in 10 mL of 0.1 N isothermal hydrochloric acid, and analyzed for chromium by the method of standard additions using a Perkin-Elmer 5000 atomic absorption spectrophotometer and Perkin-Elmer HGA-500 furnace with pyrolytically coated tubes. The contributions of the reagents and other contaminants were subtracted from all samples. Furnace conditions have been reported (Anderson and Kozlovsky, 1985). A standard addition curve was run for each tissue sample to account for matrix differences between samples. National Bureau of Standards standard reference bovine liver (SRM 1577) and International Atomic Energy Agency (IAEA) standard reference muscle (H-4) were run as checks on the accuracy of the method. Under our conditions, a mean ± standard deviation for the chromium content of bovine liver of 82 ± 6 ng/g was obtained (certified value 88 ± 12 ng/g) and 10 ± 2 ng/g for IAEA muscle standard (recommended value 10 ng/g). Citrus leaves (SRM 1572) were run as a reference standard for the basal turkey diet since the Cr concentration of citrus leaves is similar to that of the diet. Under our conditions, the Cr concentrations of SRM 1572 was 640 ± 50 ng/g (certified value 800 ± 200 ng/g).

The design of the study involving Cr concentrations of tissues was a completely randomized 2 × 4 factorial with two time periods (weeks 1 and 5) and four levels of chromium (0, 25, 100, 200 μg/g of diet). The study involving eggs was a completely randomized 4 × 3 factorial with four time periods (weeks 1–4) and three levels of supplemental chromium (0, 25, and 100 μg/g of diet). The data were analyzed by analysis of variance. The chromium and chromium by week interactions were examined by orthogonal polynomials. The level of significance used was 0.05.

RESULTS

Chromium concentrations of the various tissues following Cr supplementation for 1 and 5 weeks are given in Table I. Supplemental Cr produced small, but statistically significant increases in breast but not leg tissue (Tables I and II). Increases were too small to facilitate the use of these tissues as sources of high-Cr foods. Chromium content of the gizzard and heart increased at each level of supplemental Cr, and final concentrations, after 5 weeks of supplementation at 200 μg of Cr/g diet, were severalfold higher than those observed for turkeys consuming the basal diet. The largest increases in tissue Cr were observed in liver and kidney samples. There was a linear increase in liver Cr concentrations with increasing concentrations of dietary Cr up to 200 μg of added Cr/g of diet, both at the 1- and 5-week time points. Chromium concentrations of the kidneys increased even more than those of the liver. Kidney Cr concentrations increased from week 1 to week 5 at each of the dietary levels of Cr tested. At 200 μg of supplemental Cr, the Cr concentrations of the turkey kidney samples were more than 500 ng/g (wet weight) (more than 2000 ng/g on a dry weight basis) (Table I). There was no evidence that the dietary Cr concentrations tested were near saturating levels since there were almost linear increases in liver and kidney concentrations with

Table III. Means and Pooled Standard Errors of Cr Concentration of White and Yolk of Eggs by Week and Dietary Chromium[c]

	chromium concn,[a] ng/g				pooled
	week 1	week 2	week 3	week 4	SEM
control					
white	1.0	1.3	1.3	1.8	0.32
yolk[b]	12	11	10	15	2.0
25 ppm added Cr					
white	1.6	1.8	1.6	1.2	0.32
yolk[b]	14	15	14	14	2.0
100 ppm added Cr					
white	1.7	1.7	1.9	2.0	0.32
yolk[b]	25	26	24	29	2.0

[a] Based on wet weight, percent water for whites was 87 and 48 for yolks. [b] Significant linear increase in Cr concentration of egg yolks due to supplemental Cr during weeks 1–4. [c] Each value represents the mean of triplicate determinations of two eggs selected at random from each respective group. Large white breeder laying hens, 33 weeks of age at onset of study (hens were just starting egg production).

Table IV. Potential Cr Intake from Cr-Supplemented Turkeys

tissue	g/μg of Cr[a]	tissue	g/μg of Cr[a]
egg white	500	gizzard	83
breast	333	egg yolk	30
leg	250	liver	3
heart	91		

[a] Grams of the respective tissues that are needed to obtain 1 μg of chromium. Values calculated from turkey parts from turkeys supplemented with 200 ppm Cr for 5 weeks; values for egg whites and egg yolk are derived from turkeys supplemented with 100 ppm Cr for 4 weeks.

increases in dietary Cr. There was a significant linear Cr–week interaction for tissues from breast, heart, kidney, liver, pancreas, spleen, and lung (Table II). In each of these tissues, the increases in Cr concentration due to supplemental chromium were greater at week 5 than week 1. Increases in Cr concentration in the gizzard were similar during weeks 1 and 5 (Tables I and II).

Chromium concentrations of all of the tissues measured for the turkeys fed the basal diet were lower after 5 weeks of the study than after 1 week (Table I). Decreases were statistically significant for the leg, gizzard, and liver.

Supplemental Cr led to a significant increase in the chromium concentration of the egg yolk but not the egg white (Table III). Addition of 100 ppm Cr led to an approximate doubling of the Cr concentration of the egg yolks. Values after 1 week of Cr supplementation were similar to those during weeks 2, 3 and 4 of supplementation. Chromium concentration of the egg yolk was approximately 10-fold greater than that of the egg white when expressed on a wet weight basis and 2- to 3-fold greater on a dry weight basis for the control eggs as well as for eggs from turkeys supplemented with either 25 or 100 ppm Cr. There was no significant effect of dietary chromium on egg production.

The potential intake of Cr from edible tissues from Cr-supplemented turkeys is presented in Table IV. More than 300 g of turkey breast from turkeys supplemented with 200 ppm Cr would need to be consumed to increase Cr intake by a single microgram and more than 15 000 g of turkey breast would need to be consumed to obtain 50 μg of Cr, the minimum level of the suggested safe and adequate intake for Cr. However, only 3 g of turkey liver from Cr-supplemented turkeys would need to be consumed to increase Cr intake 1 μg and less than 150 g of liver to obtain the minimum suggested safe and adequate intake for Cr of 50 μg. While turkey liver is not a realistic food source for the general population, chromium-enriched turkey livers can be used as an experimental food for humans.

DISCUSSION

Chromium supplementation of the diet of turkey laying hens led to an increased concentration of Cr primarily in the liver and kidneys. Increases in the main edible tissues, breast and leg portions, were too low to allow these tissues to be used as suitable sources of high-Cr foods. However, the liver of Cr-supplemented turkeys appears to be a suitable Cr-enriched experimental food. After supplementation of the basal diet with 200 ppm Cr for 5 weeks, the Cr concentration of the liver was increased more than 150-fold. Therefore, consumption of approximately 150 g (5 oz) of liver/day would lead to an intake of 50 μg of Cr (the minimum suggested safe and adequate intake).

This is of particular importance for two types of studies. First, Anderson and Kozlovsky (1985) reported that the absorption of Cr is inversely related to dietary intake at normal dietary intakes of less than 40 μg/day. Absorption of Cr was approximately 2% at intakes of 10 μg/day and only 0.4–0.5% when intakes were 40 μg. Therefore, the amount of Cr excreted in the urine, which is a measure of Cr absorption, was relatively constant at approximately 0.2 μg/day. Above a dietary intake of 40 μg, absorption was roughly 0.4% and urinary Cr excretion was linearly related to dietary intake (Anderson et al., 1983). However, data for intakes above 40 μg were derived from inorganic Cr supplementation studies since there are no known foods that can be used to significantly increase Cr intake above 40 μg. However, with the advent of Cr-supplemented turkey livers, dietary Cr intake from foods can be more than doubled in experimental studies with the inclusion of 150 g of these livers/day. Second, when stable isotopes of Cr are fed to turkeys, the endogenously labeled turkey livers will enable absorption studies involving human subjects to be completed.

In a recent report (Johnson and Weaver, 1986), attempts were made to find suitable sources of intrinsically labeled dietary sources of Cr. Egg whites and yolks were tested as potential sources of intrinsically labeled food products; however, the incorporation of the labeled Cr was too low if labeled Cr was administered orally. This is consistent with our results; even at high levels of dietary Cr, the incorporation of Cr into the eggs was too low for eggs to serve as Cr-enriched foods. However, the incorporation of Cr into egg yolk was statistically significant (Table III) and may be of physiological significance.

In the study of Johnson and Weaver (1986), the incorporation of radiolabeled Cr into egg yolks was approximately 3-fold higher than the incorporation into egg whites. Similarly, the concentration of total Cr in the egg yolks was severalfold higher than that of the egg whites (Table III).

In this study, the incorporation of chromium into the turkeys was by normal physiological means and not by injection. Injection of Cr may not be a suitable method to obtain intrinsically labeled Cr tissues since injected Cr would likely not be metabolized and stored similarly to Cr that is absorbed and transported by normal means.

The levels of Cr supplementation in this study are not in the toxic range. In fact, beneficial effects of Cr on turkey poults receiving 20 ppm Cr have been reported (Steele and Rosebrough, 1981; Rosebrough and Steele, 1981). Improvements included enhanced growth rate and feed efficiency, increased rates of lipogenesis including fatty acid and glycerol synthesis and increased in vitro incorporation

of labeled glucose into glycogen.

The Cr concentration of all the tissues tested for the turkeys receiving the basal diet decreased from weeks 1 to 5 (Table I). Since all of the tissues were analyzed simultaneously and appropriate controls and standards were run as checks on the accuracy and reproducibility of the Cr analyses, these changes are an accurate reflection of physiological changes that were occurring as a result of egg production and related changes. The Cr concentrations of selected tissues of turkeys sacrificed after 4 weeks were intermediate between the 1- and 5-week values reported in this study (data not shown). The changes associated with reproduction and the added stress of egg production may be the likely reasons for the decreased Cr concentrations of the tissues from weeks one to five.

Reproduction also leads to decreased Cr stores in pregnant humans. Direct assessment of Cr status using hair chromium analysis to monitor the Cr status of pregnant and parous women has been used. Nulliparous women have significantly higher levels of hair Cr than parous women, but additional children did not further decrease Cr status as judged by hair chromium concentration (Mahalko and Bennion, 1976). Reproduction seems to be yet another stress that leads to increased Cr losses. Other stresses that may decrease Cr reserves include high-sugar diets (Kozlovsky et al., 1986), strenuous exercise (Anderson et al., 1982), physical trauma (Borel et al., 1984), acute blood loss (Mertz and Roginski, 1969), and infection (Pekarek et al., 1975).

The overall objectives of this study were to identify edible turkey parts that can be enriched with chromium and to determine food sources that can be suitably enriched with stable isotopes for human studies. Chromium-enriched turkey livers meet both of these objectives for laboratory studies involving human subjects.

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