CHROMIUM NUTRIENT INTERACTIONS AFFECTING TISSUE CHROMIUM, VITAMIN C METABOLISM, AND CHOLESTEROL SYNTHESIS

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CHAPTER I

RESEARCH PROBLEM

Introduction to Topic

Although nutrient interactions are important in human nutrition (1), chromium nutrient interactions have not been extensively studied. Because minerals often carry positive or negative charges which change based upon local pH, they can associate loosely with or bind chemically with a variety of compounds. Components in food or ingested drugs may affect mineral absorption and excretion. Intake of chromium from self-selected diets in the United States may be below the estimated safe and adequate amount of 50-200 ug chromium per day (2). Marginal intake is of concern because chromium deficiency has been linked to elevated blood cholesterol and impaired glucose tolerance (3). The extent of interaction of chromium with other nutrients is not known. However, chromium supplementation (10 ug/g diet) has increased vitamin C concentrations in adrenal and liver tissues of rats (4). Nutrient and drug interactions affecting chromium retention and utilization, the role of chromium in cholesterol metabolism, and the impact of chromium on essential nutrients are problems that warrant investigation.

Significance of Problems/Description of Experiments

Most research on interactions between minerals and carbohydrates in non-ruminants has emphasized nondigestible carbohydrates (fiber) (5). Reports of digestible carbohydrate effects on mineral absorption have involved copper (6) and iron (7). The ability of supplemental chromium to lower serum cholesterol in rats was different with diets containing sucrose versus diets containing starch (8). In humans, diets high in simple sugars (35% of calories) increased urinary chromium excretion compared to diets with 35% of calories from complex carbohydrates (9). Therefore, experiment 1 was designed to determine whether tissue concentrations of chromium and $^{51}CrCl_3$ absorption are altered either when chromium is supplemented and or when sources of carbohydrate are altered. Genetically obese mice and their lean littermates were used because obese mice exhibited insulin resistance (10) and sensitivity to Cr depletion (11).

Effects of common over-the-counter drugs on chromium status have not been investigated. The elderly may be at risk for adverse drug-nutrient interactions because of their chronic use of medications (12). Gastrointestinal tract disorders may promote antacid abuse in the elderly, and an increasing number of women have responded to recommendations for increased calcium intakes (13) by taking calcium based antacids. In inverted sac experiments, calcium appreciably depressed ^{51}Cr transport (14). Chromium formed insoluble

complexes under alkaline conditions in vitro (15). Vitamin C may facilitate the absorption of chromium by preventing the formation of insoluble chromium complexes (16). Vitamin C enhanced iron absorption (17). Therefore, experiment 2 evaluated ^{51}Cr retention and excretion in rats from $^{51}CrCl_3$ dosed concurrently either with vitamin C, with an antacid composed of calcium carbonate, or with water.

Elderly persons (18), multiparous women (19), patients receiving long-term total parenteral nutrition (20) and diabetics (21) are most likely to be at risk for chromium deficiency. Less than 2% of dietary chromium is absorbed (22). Effects of chromium status on chromium absorption and excretion have not been clarified (23), but in one study, chromium absorption (estimated by urinary excretion) was related inversely to dietary intake in human subjects (24). Cr absorption may be impaired in chronic vitamin C deficiency. In scorbutic guinea pigs the total acidity of gastric juice was decreased (25); this could reduce chromium absorption if olation and subsequent precipitation occurred in the more alkaline environment (16). Therefore, experiment 3 utilized $^{51}CrCl_3$ to evaluate chromium retention and excretion in chromium and/or vitamin C depleted guinea pigs.

Insulin is needed for the transport of vitamin C into certain tissues (26), and inadequate insulin depressed vitamin C retention in tissues (27). If chromium facilitated the transport of vitamin C as it does glucose

(28), then a chromium deficiency should affect intracellular vitamin C. In experiment 4, the effects of vitamin C and chromium status on tissue retention of ^{14}C from ^{14}C-ascorbate were determined using guinea pigs. Metabolism of ^{14}C-ascorbate was also evaluated by measuring ^{14}C in urine and expired carbon dioxide. Other effects of vitamin C and chromium status were determined by measuring tissue vitamin C, hydroxyproline, creatinine, blood urea nitrogen, cortisol and tissue minerals.

Serum cholesterol levels have been elevated in rats fed low chromium diets (29), and in chronic vitamin C deficiency, guinea pigs had lower bile acid production and higher hepatic and serum cholesterol than controls (30). Serum cholesterol elevation was exacerbated by combined chromium and vitamin C deficiencies in guinea pigs (31). Therefore, experiment 5 investigated the effects of chromium, vitamin C, and their interactions on cholesterol biosynthesis through analysis of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity.

Objectives

The following research objectives were developed:

1. To determine if type of dietary carbohydrate, either glucose, fructose, sucrose or starch, and chromium supplementation would alter tissue concentration of chromium or retention and excretion of $^{51}CrCl_3$ in obese and lean mice;

2. To determine if vitamin C or a calcium carbonate antacid would alter tissue accumulation and excretion of $^{51}CrCl_3$ in rats;

3. To investigate effects of chromium depletion and/or chronic vitamin C deficiency on tissue accumulation and excretion of ^{51}Cr from a dose of $^{51}CrCl_3$.

4. To determine if chromium depletion and/or chronic vitamin C deficiency would affect ^{14}C in plasma, tissues, urine and carbon dioxide after a ^{14}C-ascorbate dose, tissue trace minerals (copper, zinc, chromium, manganese), blood urea nitrogen, cortisol, tissue vitamin C, and urinary excretion of hydroxyproline and creatinine in guinea pigs; and

5. To determine if hepatic 3-hydroxy-3-methylglutaryl-coenzyme A activity is affected by chromium depletion and/or chronic vitamin C deficiency.

Hypothesis

The following hypothesis were developed for this study.

1. There will be no statistically significant effects of carbohydrate source (glucose, fructose, sucrose or starch), or of chromium supplementation, or genetic obesity on tissue chromium or ^{51}Cr retention and excretion from $^{51}CrCl_3$;

2. In rats dosed with $^{51}CrCl_3$ there will be no statistically significant difference in ^{51}Cr tissue

accumulation or excretion following a dosage of vitamin C, calcium carbonate antacid, or water;

3. Deprivation of chromium and vitamin C will not significantly alter ^{51}Cr appearance in tissues, blood, and urine of guinea pigs;

4. Chromium or vitamin C status will not statistically alter either ^{14}C in blood, tissues, urine and expired carbon dioxide from a ^{14}C ascorbate dose or tissue mineral concentrations, tissue vitamin C, blood urea nitrogen, cortisol, and urinary excretion of hydroxyproline and creatinine;

5. Chromium depletion and chronic vitamin C deficiency will not statistically alter hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity.

Limitations

Nutrient and drug interactions that affect chromium are difficult to assess because chromium concentrations in tissues are very low (32). Furthermore, circulating chromium may not be in equilibrium with tissue stores making it difficult to determine chromium status. Although data from animal models cannot be extrapolated directly to humans, mechanisms of chromium-drug and chromium-nutrient interactions determined utilizing animal models can help direct human research.

Format of Dissertation

Each of the five experiments was organized as an individual manuscript for publication in an appropriate journal. Chapter III was written using the Guide for Authors for the Journal of Nutrition. Chapter IV was written according to the guidelines for Nutrition Research. Chapter V was written according to directions for contributors to International Journal for Vitamin and Nutrition Research using the research note format. Chapter VI and Chapter VII were written following the guidelines of Biological Trace Element Research and Nutrition Research, respectively.

CHAPTER II

REVIEW OF LITERATURE

This chapter includes a review of absorption, excretion, and tissue distribution of the trace mineral chromium. Evidence that chromium influences cholesterol metabolism is evaluated. Metabolism of vitamin C is discussed as well as effects of vitamin C on mineral bioavailability and synthesis of cholesterol. Factors influencing hydroxyproline and creatinine excretion are reported. A review of factors affecting the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase is included.

Chromium Absorption, Excretion, Tissue Distribution

Chromium deficiency is characterized by impaired growth and longevity in experimental animals and by disturbances in glucose, lipid, and protein metabolism (29). Chromium (Cr) is a metal of the first transition series with atomic number of 24 and atomic weight of 52 g/mole. Of the five radioactive isotopes, only ^{51}Cr with a half-life of 27.8 days is available commercially for radioisotope tracer studies (33).

Absorption

The absorption of chromium (Cr) is dependent on the type of chromium complex (28). Trivalent chromium was absorbed at 0.5% compared to hexavalent chromium at 2% (22). Any chromates present in the diet appear to be reduced in the gastrointestinal tract from the hexavalent (+6) to the trivalent (+3) valence (22).

In an alkaline medium, chromium (III) is olated, i.e., forms gel-like complexes with hydroxyl and water molecules (15). Olation starts at pH values above 3.5 and increases with increasing pH and temperature (16). Ligands that increase absorption may function by preventing olation and precipitation of chromium. In rats, Chen et al (34) found that oxalate increased and phytate decreased Cr absorption both in vitro and in vivo. Chromium transport by inverted sacs was strongly stimulated by substances that formed chelates with the element (14). A mixture of amino acids doubled the rate of transport as did penicillamine. Without chelation, chromium was precipitated at alkaline pH.

Interactions with other metals may affect chromium absorption. Whole body contents of an oral dose of radioactive Cr were greater in zinc-deficient rats than in zinc-adequate controls (35). Zinc, cobalt, and chromium eluted in the same low-molecular weight fraction when mucosal supernatant extracts were separated by gel

filtration, suggesting that they combined with the same intestinal ligand.

Chromium and iron may share a common gastrointestinal transport mechanism. Iron deficient animals absorbed more Cr than iron supplemented controls and iron depressed chromium binding to transferrin (36). Transferrin also forms a complex with manganese (37). Manganese (Mn) deficiency in the guinea pig induced a diabetes-mellitus like syndrome (38) which suggested an interaction between Cr and Mn. However, in rats, supplementation with 1600 ng Cr as $CrCl_3$/g diet did not alter hepatic zinc, iron, copper and manganese concentrations (39).

Chromium absorption was greatest in the jejunum (34). Mertz and Roginski (14) used inverted jejunal sacs with $^{51}CrCl_3$ and found that the rate of chromium uptake diminished as the amount of chromium in the medium increased; this suggested that the number of absorption sites were finite. Chromic chloride uptake followed a facilitated diffusion process (14). Neither glucose, acetate, nor inhibition of cellular energy generation influenced the process. Iron, manganese, calcium, and titanium depressed chromium transport in vitro. Disodium ethylenediamine tetracetate (EDTA) in the medium overcame calcium inhibition; however, even in the absence of calcium, EDTA stimulated chromium transport.

The average daily intake of chromium from US diets is below the minimum safe and adequate recommended level of 50

to 200 micrograms per day (24). Offenbacher and co-workers reported that net absorption of Cr in subjects in a metabolic unit was 1.8% (40). But Donaldson and Barreras found an average absorption of only 0.5% of an oral dose of $^{51}CrCl_3$ in normal subjects (41). In elderly human subjects, ^{51}Cr absorption was not significantly different from young adults; but insulin requiring-diabetics absorbed two to four times more chromium than normal subjects did (21). Along with increased absorption, diabetics had increased urinary excretion of chromium (21). Streptozotocin diabetic animals retained a higher percentage of a ^{51}Cr dose in the serum than normal animals, but there were no differences in ^{51}Cr retained in the muscle, spleen, liver, heart and pancreas (42). Insulin administration increased tissue retention of ^{51}Cr in diabetic animals (43).

There is some disagreement on effects of prior chromium intake on chromium absorption. Mertz et al (23) failed to detect any influence of previous chromium intake on absorption or excretion of $^{51}CrCl_3$ in rats. Hopkins and Schwarz (44) also concluded that the percentage of chromium absorbed from an oral dose was independent of dose or chromium status of the rat. However, Anderson and Kozlovsky (24) reported that in humans chromium absorption was inversely related to dietary intake. At a dietary chromium intake of 10 micrograms, absorption (measured as urinary excretion) was approximately 2%; as intake increased to 40 micrograms, chromium absorption decreased to 0.5%.

Foods rich in chromium include brewer's yeast, meats, cheeses, whole grains, mushrooms, black pepper, nuts, and asparagus (45). Chromium in foods generally decreased with processing (46). For example, molasses contains 0.26 ug chromium/g, unrefined sugar contains 0.16 ug/g, and refined sugar contains 0.02 ug/g. As compared to whole wheat bread, white bread was 71% lower in Cr (47). However, chromium may be increased in some processed foods due to nonspecific contamination during processing (48). At high temperatures and low pH (2.5-3.0), 16 to 59 ug/g chromium was released from stainless steel cooking vessels into water; no chromium leached into unacidified water in the same vessels.

Mertz and Roginski (14) concluded that some sources of dietary chromium were more available for absorption than chromic chloride. Whether foods themselves contain substances that increase chromium retention and utilization has received little attention. Supplemental vitamin C increased hepatic iron (49) and serum iron (50) while reciprocally decreasing hepatic copper (49). Whether vitamin C increases or decreases chromium absorption has not been determined.

Excretion

Once absorbed, chromium is excreted almost exclusively via urine; therefore, Mertz (51) suggested that the measurement of urinary chromium can be used to detect gross abnormalities in chromium metabolism (51). Only very small

amounts of chromium are lost in hair, perspiration and bile (44). Anderson et al (52) suggested that, although urinary chromium excretion was not a meaningful indicator of chromium status, it is meaningful as an indicator of chromium intake and absorption. In the dog nearly all the urinary ^{51}Cr was dialyzable, and at least 63% of filtered Cr was reabsorbed (53). Ultrafilterable plasma ^{51}Cr concentration and glomerular filtration rate appeared to be the primary determinants of renal ^{51}Cr excretion (54).

Early studies indicated that glucose loading increased urinary chromium excretion in rats (15), diabetics (55), and normal humans (56), but methodological difficulties in analyzing chromium make these data difficult to interpret. One early study indicated that both glucose administration and insulin injection increased plasma chromium concentration; this may mean that the response of plasma chromium to glucose is occurring via insulin secretion (28). Kozlovsky et al (9) reported that chromium loss in 37 adults increased when they were fed diets containing 35% of kilocalories as simple sugar compared to diets containing 35% of kilocalories as complex carbohydrate. In rats, neither insulin nor glucose influenced the rate of disappearance of ^{51}Cr in blood within a few hours after an intravenous dose of $^{51}CrCl_3$, but some response to insulin and glucose was observed in ^{51}Cr disappearance from the blood after three days (14).

Liu and Morris (57) reported that serum chromium levels dropped in response to a glucose load in hypoglycemic subjects with inadequate chromium storage. In subjects receiving 5 g brewers' yeast extract containing 4 micrograms chromium daily for 3 months, serum chromium rose in response to a glucose load. Anderson et al (58) noted that urinary chromium excretion increased in response to a glucose load for nonsupplemented normal subjects but not for subjects supplemented daily with trivalent chromium.

Mertz (59) reported that when ^{51}Cr was injected into rats intravenously, approximately 14% of the dose had appeared in the feces by day 8. Contamination of feces with urine complicates quantitative assessment of fecal chromium excretion from intravenously injected chromium in animals. Fecal chromium from an intravenous dose could arise from bile, because the liver accumulates chromium. Another source is sloughed mucosal cells that had incorporated chromium (36).

Tissue Distribution of Chromium

The tissue distribution of chromium is affected by chemical form, age, species and the presence of diabetes (60). A variety of metabolic poisons strongly inhibit cellular uptake of chromium (61). Hexavalent ^{51}Cr has been used extensively as a cell label because it binds to erythocytes, tumor cells, platelets and leukocytes (61). Visek and co-workers (60) found that after intravenous

administration of ^{51}Cr as sodium chromate, almost all ^{51}Cr was localized in the reticuloendothelial system; in contrast, after administration of chromium chloride, 55% of retained ^{51}Cr was found in the liver. However, when $^{51}CrCl_3$ was buffered with acetate or citrate, most was excreted in the urine and less than 5% remained in the liver.

Kraintz and Talmage (62) injected rats intravenously with $^{51}CrCl_3$. By 24 hr, 40% of the ^{51}Cr was excreted in the urine while the bone marrow had the highest percentage of remaining activity. Although ^{51}Cr in the bone marrow was deposited in the reticuloendothelial system, it was not retained by red cell precursors.

In rats only 1.2 to 2.3% of a ^{51}Cr dose from chromium labelled foods remained in the animals by day nine (63). Hopkins and Schwarz (36) observed that ^{51}Cr from chromium chloride peaked in the blood at 30 to 60 minutes after administration by stomach tube. Whole blood values ranged from 0.07 to 0.48 percent of the dose. Even with low doses (10 to 100 ng) of chromium, chromium accumulated in the spleen (44). In humans injected intravenously with $^{51}CrCl_3$, the principal concentrations of ^{51}Cr were found in the liver, spleen, bone, muscle and adipose tissue (64). The tissues extracted ^{51}Cr very rapidly; more than 50% of plasma ^{51}Cr was taken up by the tissues within hours after intravenous administration.

In exercise trained rats, tissue chromium increased (65). In mice, age led to decreased ^{51}Cr uptake in some

tissues, specifically liver, testes, and epididymal fat pads (66). Mature rats, however, retained less ^{51}Cr in bone but more in spleen, kidney and testes than immature rats (44).

Chromium and Stress

Stress may be a major factor in chromium metabolism (14) and human chromium need (67). Stress, including trauma, infection, surgery, intense heat or cold, elevated the secretion of hormones, which altered glucose metabolism and apparently affected chromium metabolism (67). Chromium deficiency in animals has been associated with growth retardation (68), shortened life span, and decreased ability to cope with various forms of physiologic stress (69). In experimental animals, stress induced by low protein diets, controlled exercise, acute blood loss or infection increased the severity of depressed growth and decreased survival of animals fed low chromium diets (70).

Under physiological conditions, blood glucose concentrations are regulated by the hormones insulin, glucagon and growth hormone (45). Cortisol and catecholamines are elevated in various forms of stress including trauma, infection, surgery, and intense heat or cold (14). Catecholamines stimulate glucagon secretion and inhibit pancreatic insulin release (71), with a net effect of stimulating gluconeogenesis by the liver and causing hyperglycemia.

Increased serum levels of glucagon, growth hormone, catecholamines and cortisol occurred during strenuous exercise in humans (45). Anderson and co-workers (72) observed increased glucose utilization and urinary chromium excretion increased five-fold in humans two hours after running. Elderly men with ischemic heart disease had elevated serum cortisol during exercise whereas, cortisol declined in similar subjects without ischemic heart disease (73). The effects of cortisol on chromium retention and the possible link of stress to heart disease deserves further evaluation.

After trauma, plasma cortisol is elevated (45). Pekarek and co-workers reported subjects infected with sandfly fever had lower fasting serum chromium than healthy subjects (74). The mean urinary chromium concentration of traumatized patients 42 h following admission was 10 times greater than the urinary chromium concentration of normal, healthy subjects (75). Effects of cortisol on tissue distribution of chromium have not been studied, but circulating plasma cortisol has been linked to stress related increases in sodium and calcium excretion in the rat (76). Increased zinc and chromium excretion have been reported in human runners with elevated plasma cortisol (77).

Measurement of changes in chromium metabolism as a result of stress is difficult. Currently there are no established biochemical indices of chromium status; confirmation of human chromium deficiency is dependent on

beneficial effects of dietary chromium supplementation. Chromium deficiency in man causes glucose intolerance, neuropathy, high free fatty acid concentrations, and abnormalities of nitrogen metabolism (20,78), but these symptoms are not specific enough to be used as chromium status indicators. Therefore, a clinical measure of chromium status is needed.

Chromium and Cholesterol

Chromium supplementation has resulted in lowered blood cholesterol and chromium deficient diets have produced elevated cholesterol in animals (8). However, mechanisms for these effects are speculative. Chromium may affect cholesterol through effects on synthesis and/or degradation of cholesterol.

Evidence that chromium functions in the biosynthesis of cholesterol is limited. Using labelled acetate, Curran (79) was the first to demonstrate that chromium enhanced the synthesis of cholesterol and of fatty acids in rat liver. When chicks were fed 20 ug Cr/g diet, the chromium increased ^{14}C acetate incorporation into hepatic fatty acids compared to the controls (80). Xu and co-workers reported increased plasma cholesterol in guinea pigs supplemented with 4 ug Cr from $CrCl_3$/g diet versus 2 ug Cr/g diet (81). Yet chromium has not been implicated in the function of 3-hydroxy-3-methylglutaryl coenzyme reductase, the rate limiting step in cholesterol biosynthesis.

Mertz and co-workers found that chromium from glucose tolerance-like compounds enhanced glucose uptake by epididymal fat tissue of rats (82). Glucose uptake by adipocytes from chromium deficient rats did not differ from controls in the absence of insulin; however, addition of chromium in vitro or in vivo to the donor animals increased the response of the tissue to exogenous insulin (59).

Serum cholesterol was elevated and increased with age in rats receiving sucrose; in rats given sucrose plus Cr serum cholesterol levels were lower (83). Feeding 1 ug/g chromium decreased serum cholesterol in male rats, while 5 ug/g Cr lowered serum cholesterol in female rats (84). Potassium chromate injected intraperitoneally reduced the size of aortic plaques and decreased aortic cholesterol in rabbits fed a high cholesterol diet (85). However, Preston and colleagues (86) demonstrated that addition of 0.5 ug Cr/g diet for 21 weeks had no effect on serum cholesterol of pregnant guinea pigs.

Total hepatic lipid was significantly lower in obese mice fed chromium supplemented (2 ug Cr as $CrCl_3$/g) diet compared to chromium depletion diets (11). In male rats fed 5 ug Cr/g diet, the decrease of circulating cholesterol with normal levels of aortic lipids suggested that chromium may function in the catabolism of cholesterol (87). In female animals, comparable changes in serum cholesterol were not observed, which suggests sex differences in response to Cr supplementation (87).

Schroeder et al (88) found decreased chromium in aortic tissue from individuals who died of atherosclerotic heart disease compared to those who died in traffic accidents. Although problems with chromium measurement make interpretation of the data difficult, these authors hypothesized chromium functions in the catabolism of cholesterol and may have a basic role in atherosclerosis.

In a double-blind 12 week study of 23 men (31-60 yr of age) supplemented with 200 ug trivalent Cr in water, high density lipoprotein cholesterol increased in the Cr supplemented group (89). This study was well controlled with half of the subjects ingesting a water placebo. Several studies which have reported cholesterol lowering effects of supplemental chromium have lacked a placebo group. When twenty-four non-insulin-dependent diabetics were fed fruit juice containing either chromium-rich Brewers yeast or chromium-poor Torula yeast (the control) (90), cholesterol decreased ($p<0.001$) with the chromium-rich Brewers yeast. Elwood et al (91) gave 20 g Brewers yeast to 11 adults with normal lipids and 16 adults with hyperlipemia and reported a 10% and 9% reduction respectively in the two groups.

Not all studies have found a decrease in total cholesterol as a result of chromium supplementation. When Offenbacher and co-workers supplemented non-institutionalized elderly volunteers with 200 ug $CrCl_3$, 5 g Brewers yeast, or placebo for ten weeks, no changes in blood

cholesterol or triglycerides were noted (92). When Riales and Albrink (89) supplemented 12 healthy adults with 200 ug Cr, blood cholesterol did not change; however, there was a 12% increase in high density lipoprotein (HDL) cholesterol. Grant and McMullen (93) supplemented 37 non-insulin dependent diabetics with 1.6 g brewers yeast and reported no change in total cholesterol, but HDL cholesterol increased by 36%. Polansky et al (94) using 200 ug Cr as $CrCl_3$, 8 g Brewers' yeast or placebo during three separate three month periods found no change in total cholesterol in 30 adults with normal oral glucose tolerance tests.

The conflicting results obtained in these supplementation experiments may indicate that diets of subjects not responding to chromium supplementation were adequate or that cholesterol elevation was not present prior to supplementation. These results could further reflect dietary factors and other physiological factors, such as stress, not controlled in these experiments.

Individually, chromium (83) and vitamin C deficiencies (30) have contributed to elevated serum cholesterol. Studies in guinea pigs have shown that the cholesterol elevation is exacerbated by combined chromium and vitamin C deficiencies (31). Synergistic effects of chromium and vitamin C need evaluation as do mechanisms by which chromium supplementation may affect cholesterol metabolism.

Vitamin C Metabolism

Vitamin C, $C_6H_6O_8$, is a six carbon compound with a molecular weight of 176 (95). A critical function of vitamin C is its ability to donate electrons while itself undergoing reversible oxidation to dehydroascorbic acid which has a half-life of only a few minutes (96). Metabolic functions of ascorbic acid include hydroxylation of dopamine to form norepinephrine, hydroxylation of peptidyl proline to form hydroxyproline, and enhanced secretion of procollagen (95).

Absorption

In guinea pigs, vitamin C was absorbed primarily in the duodenum, whereas in the rat the greatest absorption was in the ileum (97). In intestinal tissue from guinea pigs vitamin C was transported by a sodium-dependent active transport system, but the transport of dehydroascorbic acid was by simple diffusion (98). In addition to the carrier-mediated process that was saturated at low ascorbic acid concentrations, ascorbate entered the tissue from the intestinal lumen by simple diffusion (99).

Ascorbate transport across the brush border in the guinea pig small intestine was inhibited by D-glucose (100) and aspirin (101). Ascorbic acid accumulated in mucosal cells of scorbutic guinea pigs indicating impaired intestinal absorption (102). Likewise, the transport of

ascorbic acid in vitro was significantly reduced in brush border membranes from scorbutic animals (103).

Ascorbic acid was reabsorbed in the kidney by a sodium-dependent active transport mechanism that operates by concentrating ascorbic acid in the cellular fluid. Both rat and guinea pig kidneys handled ascorbic acid similarly (104). Rose (105) suggested a cytosolic enzyme functions in reduction of dehydroascorbic acid to maintain the redox state of ascorbic acid in the process of reabsorption in the kidney. Otsuka and co-workers (106) reported that utilization of injected dehydroascorbate in vitamin C deficient guinea pigs was less than in control animals and suggested that the reduction mechanism of dehydroascorbic to ascorbate was impaired in guinea pigs deficient in vitamin C.

Distribution

The tissue distribution of vitamin C is similar in man and other species dependent upon dietary intake. The highest concentrations were observed in glandular tissues (pituitary gland, adrenal glands, corpus luteum, and salivary glands), whereas the vitamin C concentration in muscle tissue was relatively low. Pancreas, liver, lungs, brain, spleen, thymus, and kidneys were intermediate (107). Damron and colleagues (108) determined that dehydroascorbic acid and 2,3-diketogulonic acid were present in guinea pig tissues only in negligible concentrations.

Ginter and co-workers (109) reported that guinea pigs fed ascorbic acid in the diet had higher ascorbate in the tissues than animals where ascorbic acid was administered orally once a day. There was a linear relationship between ascorbic acid concentrations in the tissues and the graded oral dose (110). Five and ten minutes after intravenous injection of ascorbic acid, the pituitary and adrenal glands, spleen, pancreas and parotid glands had the highest accumulation of label as visualized by autoradiography (111). Uptake of ascorbate was significantly greater in platelets of ascorbate deficient guinea pigs than in controls (112). In guinea pigs fed a scorbutogenic diet, the loss of ascorbate from the brain and lens was slower than the loss from the adrenal glands and spleen (113).

In Rhesus monkeys, plasma ascorbate was directly related to liver ascorbic acid levels ($r=.48$, $p<0.05$) but leukocyte ascorbate was the best indicator of the vitamin C status (114). Veen-Baigent and colleagues (115) suggested that ascorbic acid may not be freely exchangeable among tissues or between blood and tissues. At very low levels of intake, tissue ascorbic acid rose at the apparent expense of leukocyte ascorbic acid. The data were suggestive of a preferential transfer of vitamin C from blood (including leukocytes) to tissues supporting essential ascorbic-acid dependent functions when ascorbic acid intakes were low (0.05 to 0.15 mg/100 g body weight). At adequate intakes (0.5 to 2.0 mg/100 g body weight), blood and some tissues

(kidney, heart, and spleen) remained relatively constant as liver and adrenal ascorbic acid increased. Above 2.0 mg/100 g body weight, ascorbic acid in all tissues rose until saturation was attained.

Half Life

From depletion experiments in guinea pigs, Pelletier (116) calculated the half-life of 1-^{14}C-ascorbic acid in the liver, heart, kidneys, adrenals and spleen to be 2-3 days, whereas the brain was about 5 days. Ginter et al (117) found the time for ascorbate depletion in nine organs to be similar in guinea pigs receiving 0.5 mg/day and approximately 150 mg/day. The half-life of ascorbic acid was shorter in tissues of younger guinea pigs; younger animals were depleted much more rapidly than older animals (118).

The half-life of ascorbate in the guinea pig (4 days) was much shorter than in humans (16 days) (119). Likewise, a larger dose of ascorbic acid was required to maintain an equivalent body pool of the vitamin in the guinea pig than in humans (119).

Transport

The active transport of ^{14}C-labelled ascorbic acid into rat brain cortex and ovary slices was inhibited by corticosteroids, estrogen, and testosterone, but uptake was not affected by ACTH (120). There was no active uptake by

ascorbate uptake (126); however, equimolar concentrations of fructose did not inhibit uptake. The absence of insulin also significantly reduced ascorbic acid uptake. Kapeghian and Verlangieri (27) confirmed that ascorbic acid uptake by the cell was compromised by decreased insulin and/or increased extracellular glucose levels.

Bianchi and Rose (1986) (127) demonstrated in vitro a facilitated diffusion pathway of dehydroascorbic acid transport in human erythrocytes that was not influenced by external glucose. Physiological levels of D-glucose limited dehydroascorbic transport on the hexose transporter as proposed by Mann and Newton (122), but high levels of glucose did not interfere with transport of dehydroascorbic by the alternate pathway. Mooradian (128) studied brain and muscle uptake of glucose with various concentrations of ascorbate and dehydroascorbate. Dehydroascorbic acid, but not ascorbic acid, inhibited in vivo uptake of glucose by brain, but not by muscle which suggested a common carrier system at the level of the blood-brain barrier.

Catabolism

The metabolic fate of ascorbic acid and its derivatives in animals depends on a number of factors including species, age, route of administration, quantity of material and nutritional status (129). Urinary ascorbate excretion increased in older compared to younger guinea pigs (130),

and animals fed lower ascorbic acid had a higher retention of a radioactive dose (130).

The major pathway of catabolism of ascorbic acid in the guinea pig is the oxidation of its lactone carbonyl carbon to carbon dioxide with subsequent oxidation of the entire carbon chain to carbon dioxide (131). A biphasic pattern of expired ^{14}C-carbon dioxide evolution has been noted in guinea pigs; the first peak occurred at two hours and the second peak at five hours after administration of the dose (132). The in vivo catabolism of 1-^{14}C-ascorbate to carbon dioxide was related to the route of administration (132).

Dehydroascorbic acid is a major intermediate in the breakdown of ascorbate, and L-xylose and oxalate are formed as subsequent products (133). A ^{14}C-labelled excretory product, ascorbic acid-2-sulfate, has been identified in excreted bile (134), but Kipp and Rivers found less than 1% of a ^{14}C-ascorbate dose in the feces (135). Incorporation of ^{14}C in expired carbon dioxide, urinary oxalate and liver glycogen was via dehydroascorbic acid and diketogulonic acid (136).

In the guinea pig, 66% of the ^{14}C from a dose of 1-^{14}C-ascorbic acid was excreted as carbon dioxide, 10% was in the urine and less than 1% of the administered radioactivity appeared in the feces during the ten days following injection of the isotope. Approximately 5 to 24% of the dose was catabolized to carbon dioxide during the first 6 h; 30-40% at 24 hr (137). Burns et al (137) noted no

difference in total excretion (formation of carbon dioxide and urinary metabolites) in scorbutic guinea pigs and in those fed 2, 15, and 50 mg ascorbate daily. However, Schuching and co-workers (138) found that guinea pigs fed the highest level of ascorbic acid exhaled the most labelled carbon dioxide. The amount of ^{14}C-carbon dioxide exhaled after administration of 1-^{14}C-ascorbic acid was dependent on the previous dietary intakes of vitamin C. The lower the ascorbic acid intake, the lower the ^{14}C-carbon dioxide excretion (138).

Guinea pigs metabolized ^{14}C-ascorbic acid much faster to ^{14}C-carbon dioxide (peak exhalation at 30 min) than rats (peak exhalation at 2 to 3 h) following a single oral dose (139). Urinary excretion of ^{14}C in rats and guinea pigs was approximately 5-10% of the administered ^{14}C-ascorbate dose during the first 24 h and 21-22% after 48 h (140). Unchanged 1-^{14}C-ascorbate, dehydroascorbic, 2,3-diketogulonic acids (2-3% of the dose), and ^{14}C-oxalate (7% of the dose) were isolated (140).

Hormones and Vitamin C Metabolism

In humans, ascorbic acid needs of individuals vary widely due to changes of metabolism, hormonal activities, and stages of development (141). In guinea pigs deprived of food to produce a mean weight loss of 246 g in 16 days, vitamin C retention of adrenals, spleen, liver, but not brain decreased (142). Older animals had lower tissue

ascorbate concentrations than young ones (143). Davies et al (143) suggested that the difference in ascorbate saturation was a reflection of a reduced capacity of "aged" tissue to abstract or retain ascorbate.

Compounds dependent on the pituitary gland may influence ascorbate uptake by various tissues in the rat; a lower uptake of a single orally administered dose of 1-^{14}C-ascorbate was observed in hypophysectomized rats, but concentration in circulating blood was higher (144). Horning et al (144) suggested participation of pituitary hormones in regulating the transport, tissue binding or catabolism of vitamin C.

Scorbutic guinea pigs had decreased pancreatic insulin. Insulin administration corrected the abnormal glucose tolerance in the scorbutic animals (145). In scorbutic animals, blood ketone bodies were elevated (146). Urinary excretion of vitamin C decreased in rats injected intraperitoneally with ketone bodies, and the ketone bodies brought about a significantly high destruction of vitamin C (147).

Increasing concentrations of insulin increased transport of radioactive vitamin C into fetal bovine heart endothelial cells. A linear relationship was found between the log of the insulin concentration and the uptake of vitamin C. In media containing 180 mg/dl glucose, there was approximately a five-fold decrease in vitamin C uptake which Verlangieri

and Sestito (126) attributed to the competition for insulin receptor sites.

Acute vitamin C depletion in guinea-pigs was accompanied by increased uptake of ^{131}I by the thyroid in vivo (148). However, as vitamin C deficiency progressed, there was subsequent inanition and weight loss. Under these conditions the uptake of ^{131}I in vitro was decreased (149).

Increased peripheral de-iodination of thyroxine (T_4) and triiodothyronine (T_3) has been reported in guinea pigs fed a low vitamin C diet (rabbit ration) (150). Added ascorbate strongly inhibited thyroxine (T_4) deiodinase of rat liver microsomal homogenate and thus protected T_4 from excessive degradation (151). Ascorbic acid and dehydroascorbic acid in liver and kidney of thyroidectomized rats decreased and total vitamin C in urine increased (152). In hyperthyroidism, plasma ascorbic acid content was reduced as well as urinary excretion of ascorbic acid (153).

Stress may affect tissue and plasma levels of vitamin C. Chronic immobilization stress decreased vitamin C in serum, liver, adrenal glands, and urine of rats (154). Other researchers reported increased serum ascorbate in rats subjected to acute immobilization stress (155). Adrenal vitamin C decreased gradually with a marked accumulation of corticosterone in the rat tissues (155). The physical stress of swimming resulted in decreased vitamin C concentrations in the adrenal glands, spleen and brain of guinea pigs (156).

Higher plasma corticosteroids were found in vitamin C deficient guinea pigs (157). Odumosu and Wilson (158) reported that as vitamin C concentrations in adrenals increased in surviving guinea pigs deprived of vitamin C, plasma cortisol concentrations diminished. Adrenal vitamin C may modulate production of adrenal steroids during stress in guinea pigs (159). Adrenal vitamin C falls as cortisol secretion into the plasma increases.

Adrenalectomy affects the degradation of ascorbate and its concentration in selected tissues (160). Adrenalectomized rats excreted significantly less radioactivity from ^{14}C-ascorbate resulting in a longer estimated half-life of ascorbate than in the sham-operated controls. The concentration of ascorbate was significantly higher in the heart and lower in the liver of adrenalectomized animals.

Elevated adrenal corticosteroids were found in guinea pigs fasted for 3 days (161). Elevated plasma corticoids were also found in non-fasted animals ingesting massive amounts of ascorbate; ascorbic acid may competively inhibit catecholamine inactivation (162).

Interactive Effects of Vitamin C and Minerals

The role of several metal ions in alterations of tissue levels and urinary excretion of vitamin C in rats has been studied (4). Chromium or tungsten at 5 ug/g diet increased the concentration of vitamin C in liver and adrenals of rats

(4), and chromium at 10 ug/g diet increased concentration of vitamin C in the spleen and adrenals of rats and the spleens of chicks (163). Lower brain vitamin C was reported in brindled mice, which exhibit signs of copper deficiency. Injection of cupric chloride into pups deficient in copper raised brain ascorbate and returned catecholamine levels to normal (164). Zinc supplementation decreased the urinary excretion of ascorbate (165) and administration of molybdenum, cadmium, or mercury decreased hepatic vitamin C of rats (4).

The effects of vitamin C on the absorption and retention of several minerals have been reported. Solomons and co-workers (166) found vitamin C over a range of dosages commonly consumed by man had no demonstrable effect on the absorption of zinc sulfate. In male guinea pigs supplemented daily with excess vitamin C (25 mg/100 g bw per day), two-to-three-fold decreases in liver copper compared to controls were noted (167). Serum and hepatic iron levels also increased as the vitamin dose increased. Smith and Bidlack (49) reported similar results in female guinea pigs. Very high dietary vitamin C (225 mg/animal) increased tissue vitamin C levels, increased hepatic iron stores, and produced a reciprocal decrease in hepatic copper. Finley and Cerklewski (168) confirmed that vitamin C intake of 500 mg/day is antagonistic to copper status of adult men as had been demonstrated in laboratory animals.

In guinea pigs, administration of vitamin C (5 mg/100 g bw) along with 5 mg of iron sulfate not only counteracted the toxic effect of decreased growth and mortality of the iron supplement, but also led to a greater utilization of iron as revealed by increased hemoglobin and serum iron levels (50). Iron administration increased vitamin C destruction as revealed by lower urinary, blood, and tissue levels of the vitamin (50). Tissue ascorbate tends to be low in iron overloaded human patients (169).

In summary, vitamin C functions to donate electrons while itself undergoing reversible oxidation to dehydroascorbic acid (96). In guinea pigs, the major pathway of catabolism of ascorbic acid is oxidation to carbon dioxide (131). Active uptake of ascorbate was demonstrated in brain and ovaries but not in liver, spleen, testes, pancreas, and diaphram (120). Uptake of vitamin C into tissues was compromised by decreased insulin and/or increased extracellular glucose levels (27). Stress decreased tissue and plasma ascorbate levels of guinea pigs which illustrated the orchestration of hormonal control on ascorbate metabolism (156). The effects of ascorbic acid on absorption of most minerals have not been thoroughly investigated; the experiments thus far have used extremely high ascorbate doses. The effects of smaller ascorbate doses on mineral status also need evaluation. Due to the frequency of ascorbate supplementation by humans,

ascorbate/mineral interactions are an ongoing concern in human nutrition.

Vitamin C and Cholesterol

In guinea pigs with chronic ascorbic acid deficiency the catabolism of cholesterol to bile acids was significantly decreased (30). Cholesterol accumulated in serum and in liver of guinea pigs with chronic latent vitamin C deficiency. Hepatic ascorbic acid concentration was negatively correlated ($p<0.001$) with cholesterol of liver and serum (170). Elevated cholesterol in ascorbate-deficient animals was reported to be due to the decreased rate of transformation of cholesterol to bile acids (171). Supporting this suggestion, acutely scorbutic guinea pigs injected with ^{14}C-cholesterol labelled in the 26 position of the side chain exhaled subnormal amounts of $^{14}CO_2$ (171).

Shefer and co-workers (172) were the first to report that regulation of bile acid biosynthesis was exerted in vivo via cholesterol 7 alpha-hydroxylase. Bjorkhem and Kallner (173) assayed cholesterol 7 alpha-hydroxylase activity in liver microsomes from guinea pigs supplemented with ascorbate and from ascorbate-deficient guinea pigs (173). The 7 alpha-hydroxylation was markedly reduced in the ascorbate-deficient animals. Chronic ascorbate deficiency was associated with a decline of hepatic ascorbic acid concentration; the rate of cholesterol transformation

to bile acids was reduced resulting in cholesterol accumulation in plasma and liver of deficient animals (174).

Holloway and Rivers (175) and Peterson and co-workers (176) demonstrated the susceptibility of cholesterol 7 alpha-hydroxylase activity in guinea pigs to both inadequate and excessive dietary extremes of ascorbate. Both deficient and excess groups exhibited lower cholesterol 7 alpha-hydroxylase activity, lower bile acid turnover rate and increased plasma and liver cholesterol.

Male guinea pigs fed a scorbutogenic diet supplemented with 1 mg ascorbate per animal per day responded with high blood cholesterol. Those animals fed 500 mg ascorbate/kg diet or 5000 mg ascorbate/kg diet displayed decreased blood cholesterol, but guinea pigs fed excess ascorbate (20,000 mg/kg diet) demonstrated blood cholesterol not significantly different from the ascorbate deficient group (177).

Hornig and Weiser (178) reported that cholesterol oxidation was dependent on the ascorbic acid status in depleted animals, but could not be further stimulated by ascorbic acid in animals fed adequate amounts of the vitamin. These data suggested that ascorbate has a cholesterol lowering effect only over a limited range of ascorbic acid intakes.

Human studies of ascorbate supplementation have produced conflicting results which appear to be related to ascorbic acid status. Young female volunteers (21-28 yr), all meeting the Recommended Dietary Allowance for ascorbate,

were given 1 g ascorbate/day for 4 wk (179). No effect was found on plasma lipids or lipoprotein cholesterol. In a long term experiment in 82 older adults, 1 g ascorbic acid/day reduced blood cholesterol values and these effects persisted 6 wk after termination of the experiment (180). The average intake of ascorbate per day for these elderly subjects was 20 mg. A similar decline in serum cholesterol was found in patients with non-insulin dependent diabetes mellitus who were supplemented with 500 mg ascorbate per day for 12 months (181). Before treatment, ascorbate concentration in blood and leukocytes of these diabetic subjects was substantially lower than in healthy subjects.

In guinea pigs a short term ascorbate deficiency (10 days) reduced both excreted bile acid and bile acid pool size by 50% (182). Cholesterol 7 alpha-hydroxylase activity also was decreased in the deficient animals. In these studies, short-term ascorbate deficiency impaired bile acid metabolism independent of any apparent side effects of clinical scurvy. In contrast, Holloway and Rivers (183) found prolonged consumption (7-9 wk) of inadequate or excessive ascorbate by guinea pigs resulted in little or no change in bile acid metabolism and biliary lipid composition except that bile acid pool size was increased 12% as a result of excessive ascorbate ingestion. Therefore, there may be important differences in metabolic response to ascorbic acid deficiency depending on the length of the experimental period or some other aspect of the experimental

protocol, i.e., Holloway and Rivers administered ascorbate in 1 ml of 5% glucose solution.

In guinea pigs the predominant bile acid in gall bladder bile and in the small intestine was chenodeoxycholic acid (184); 89-90% of total cholesterol was converted to chenodeoxycholic acid (185). In guinea pigs with chronic marginal vitamin C deficiency, the activity of the cholesterol 7 alpha-hydroxylating system was depressed resulting in cholesterol accumulation in the liver, plasma, and arteries and in cholesterol gallstone formation (180). Ascorbate administration to deficient guinea pigs enhanced cholesterol hydroxylation at the seven position resulting in the formation of 7 alpha-hydroxycholesterol (185).

Jenkins (186) reported that gallstone formation in vitamin C deficient guinea pigs fed a 0.5% cholesterol diet was associated with qualitative changes in the gallbladder bile including a high cholesterol concentration, a lowered bile acid content and diminished phospholipid-to-cholesterol and bile acid-to-cholesterol ratios. Jenkins (187) concluded that, in the chronically ascorbate depleted animal, there was a reduction in gallbladder bile acid and impaired conversion of cholesterol to chenodeoxycholic acid which favored cholesterol precipitation and gallstone formation.

Feeding rabbits a 0.3% cholesterol diet resulted in atherosclerotic fatty streaks most pronounced in the aortic arch and a decrease in the prostacyclin production by the

aortic endothelium. Addition of ascorbic acid resulted in decreased lipid infiltration and intimal thickening with a tendency to restore the prostacyclin output (188). Ginter and Ondreicka (189) maintain that the effects of vitamin C on cholesterol metabolism may be hormonal effects on enzyme systems involved in the synthesis or catabolism of cholesterol.

Banerjee and Singh (190) found total body cholesterol content increased in scorbutic guinea pigs in comparison with normal controls. However, treatment of scorbutic animals with insulin lowered the cholesterol content to that of controls. Cholesterol content of the intestines also increased as a result of ascorbate deficiency and was lowered with insulin.

Only a few researchers have suggested that ascorbic acid affects cholesterol levels by metabolic pathways unrelated to cholesterol 7 alpha-hydroxylase. Terada et al (191) reported that the hypolipidemic and lipotropic effects of ascorbate were due to sulfation of cholesterol by ascorbic acid 2-sulfate and the resultant fecal excretion of cholesterol sulfate. Durr and Shukairy (192) proposed that ascorbate stimulated synthesis of nonsteroidal compounds thus diverting mevalonate into pathways other than cholesterol synthesis. Holloway and co-workers (193) found that the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (the rate limiting step in cholesterol

biosynthesis) was significantly reduced in guinea pigs fed either inadequate or excessive dietary ascorbate.

To summarize, in ascorbic acid deficient guinea pigs, reduced activity of cholesterol 7 alpha-hydroxylase (194) and decreased catabolism of cholesterol to bile acids (30) resulted in cholesterol accumulation and gallstone formation (187). However, the negative effects of both high and low ascorbate intakes on 7 alpha-hydroxylase may be more evident in short-term versus long-term ascorbate depletion (182)(183). Ascorbate status also affected cholesterol synthesis (193) and fecal excretion as cholesterol sulfate (191).

Hydroxyproline

Hydroxyproline is found almost exclusively in collagen in the tissues of vertebrates. Except for the small amount of hydroxyproline in elastin and complement component Clq, no animal protein except collagen contains significant amounts of this amino acid. Urinary hydroxyproline reflects changes in the metabolism of collagen in various experimental conditions and disease states. Conditions which alter hydroxyproline excretion include abnormalities of growth (195), and thyroid disorders (196), as well as bone conditions including osteoporosis (197), rheumatoid arthritis (198), osteomalacia (199) and rickets (200).

Hydroxyproline and hydroxylysine in collagen arise through the hydroxylation of prolyl and lysyl residues after

they have been incorporated into peptide linkage. Hydroxylation appears to occur primarily while the polypeptide chain is being formulated and is therefore still attached to the ribosome (201).

Ascorbic acid may have three functions in collagen synthesis. Ascorbate supplied hydrogen equivalents for the hydroxylation of proline residues to form 4-hydroxyproline (202), it reactivated proly-4-hydroxylase by reducing the enzyme-bound Fe (III) (203) and promoted collagen production by a specific stimulation of procollagen mRNA (204). Nemethy and Scheraga (205) demonstrated that hydroxyproline stabilized not only the triple-helical collagen molecule but also its assembly into microfibrils.

Hydroxyproline produces no known intermediates of physiological utility and its energy contribution is insignificant (206). In animal tissues a pathway from hydroxyproline to pyruvate exists for oxidizing the 250 mg (or more) of free hydroxyproline arising daily from collagen turnover (206).

Urinary Excretion of Hydroxyproline

Urinary excretion of 4-hydroxyproline has been used as an index of whole-body collagen degradation. As a means of non-invasive quantitation of whole body bone resorption, the excretion rate of hydroxyproline appeared comparable with isotopic methods in patients with primary osteoporosis (207).

When the hydroxyproline in collagen was labeled by administering radioactive proline, changes in the excretion of labeled hydroxyproline made it possible to distinguish between the rate of collagen synthesis and the degradation of soluble collagen or of insoluble collagen (208). The differences in ^{14}C-hydroxyproline excretion between young and old animals suggested that in young animals most urinary hydroxyproline originated from the degradation of soluble collagen, while in older animals the major part of urinary hydroxyproline originated from the degradation of insoluble collagen. It is estimated that only 5-10% of the hydroxyproline released by the degradation of insoluble collagen under normal conditions is recovered as hydroxyproline in urine. Therefore, relatively large changes in collagen metabolism are required to produce significant changes in the amount of hydroxyproline excreted in urine.

Total body content of insoluble collagen increased more rapidly with age than did body weight. Thus even though total soluble collagen remained unchanged or increased somewhat, the ratio of soluble collagen to insoluble collagen decreased. One hundred week old rats had more than twenty times as much collagen as three week old rats, but their hydroxyproline excretion was only one-third that of younger rats. Collagen pool sizes increased while the amount of collagen degraded decreased; therefore, the

percentage of collagen degraded decreased markedly as animals grew older (200).

When growth hormone was administered to animals, synthesis of soluble and insoluble collagen increased as did the amount of hydroxyproline excreted (200). Biochemically, hydroxyproline is related to growth; more hydroxyproline was excreted by children and young animals than by adults and old animals (209).

Increased urinary hydroxyproline is an indicator of bone growth in health and the turnover of structural protein within bone (195). Stolley and co-workers (210) found a close correlation between urine hydroxyproline excretion and growth velocity in children. In children suffering from kwashiorkor and marasmus, hydroxyproline excretion is depressed (211).

In young rats a protein and calorie deficiency markedly reduced urinary hydroxyproline within 1 wk of feeding the deficient diet (212) (213). Protein deficiency affected urinary excretion of hydroxyproline more severely than calorie deficiency and might be explained by slow turnover of collagen in these animals (212).

Hydroxyproline in Vitamin C Deficiency

In scorbutic guinea pigs, proline in collagen was 5-10% less hydroxylated than that normally occurring in collagen (214). Barnes et al (215) likewise suggested that ascorbic

acid deprivation decreased hydroxylation of proline in elastin resulting in a polymer deficient in hydroxyproline.

Bates (216) reported that the age-related increase in hydroxyproline:creatinine ratio in young guinea pigs was significantly larger in vitamin C-deficient animals than in pair-fed controls. He proposed that the increase in hydroxyproline excretion in vitamin C-deficient animals represented the rapid turnover of underhydroxylated collagen. Degradation of soluble collagen normally accounts for a disproportionately large share of urinary hydroxyproline, thus an increase in the proportion of soluble collagen could in itself explain an increase in hydroxyproline excretion (200). Of several tissues examined for collagen synthesis, the greatest effect of vitamin C deficiency was observed in skin (216).

Not all researchers have found an increased excretion of hydroxyproline in scurvy. Robertson and Hewitt (217) found decreased urinary hydroxyproline in scorbutic guinea pigs. They suggested that when collagen synthesis was depressed, both the amount of soluble collagen and the amount of collagen degraded were decreased. Barnes and colleagues (218) reported a decreased urinary excretion of total amino acids and hydroxyproline in scorbutic guinea pigs commencing at approximately the fifteenth day of vitamin deficiency. A reduction in hydroxyproline excretion in guinea pigs after 21 days of ascorbic acid deprivation compatible with reduced

collagen synthesis and reduced excretion of hydroxyproline has also been reported (219).

Hormonal Effects on Hydroxyproline

Schneir and co-workers (220) characterized degradation products of recently synthesized collagen in skins of control and diabetic rats killed after ^{3}H-proline injection. Free and peptidyl ^{3}H-hydroxyproline were dramatically enhanced by streptozotocin-induced diabetes. They suggested the source of the degradation products was procollagen.

Cortisone produced changes in collagen metabolism similar to those sometimes seen in scurvy. The principal effect of cortisone or cortisol is to decrease collagen synthesis (221). In two-month old rats the concentration of free hydroxyproline in the serum and the excretion of free and total hydroxyproline in the urine decreased significantly after the administration of cortisone (222). By contrast, in seven-month-old rats, no changes were observed after the administration of cortisone (222). Similar results were found with administration of pharmacological doses of cortisone, cortisol, or other glucocorticoids. The excretion of hydroxyproline in urine was markedly reduced in young rats, whereas in older rats no effect or only a tendency to decrease was found (223).

Early studies in rats indicated that urinary excretion of hydroxyproline was greatly increased in experimental hyperthyroidism and decreased in experimental hypothyroidism

(224). In hypothyroid animals, hydroxyproline excretion decreases to less than one-half of control values. The decreased hydroxyproline excretion is secondary to both decreased collagen synthesis and decreased collagen degradation (224).

In summary, hydroxyproline is found almost exclusively in collagen. Measurement of hydroxyproline has been the most useful in abnormalities of bone metabolism (200). Hydroxyproline stabilizes the collagen molecule (205), and the increased hydroxyproline excretion sometimes reported in scurvy has been attributed to a rapid turnover of underhydroxylated collagen (216). Not all researchers have reported increased hydroxyproline excretion in guinea pigs with scurvy (217). Therefore, vitamin C deficiency may not result in increased hydroxyproline excretion in guinea pigs.

Creatinine

Most creatine is found in skeletal muscle where it exists both as creatine and as creatine phosphate; creatine phosphate is converted to creatine in muscle with synthesis of ATP (225). Creatinephosphokinase was inhibited by a low vitamin C diet which is of physiological significance since activity of creatinephosphokinase is closely associated with the contractile function of muscle (226). Creatine is synthesized extramuscularly and then is transported to muscle. Both creatine phosphate and creatine undergo a nonenzymatic irreversible dehydration to form creatinine.

Unlike creatine, creatinine is not retained by muscle but is distributed in total body water and cleared from the body by the kidney (225).

Heymsfield and co-workers (227) reviewed the validity of urinary creatinine as an estimator for muscle mass. Parallelism between total body creatine and urinary excretion of creatinine has been reported in a variety of species. Stress increased creatinine output. Mice exposed to cold stress demonstrated increasingly greater plasma creatinine levels with longer exposure times compared to non-exposed controls (228). Creatinine excretion increased with growth (229) and declined with aging which presumably reflected the diminution of muscle mass (227). Creatinine measurement is probably not a valid measure of muscle mass in the presence of stress such as infection, fever or trauma which increased its excretion (227). In diabetics with renal disease there was decreased excretion of creatinine when the glomerular filtration rate fell (227).

Creatinine and Hormones

Asplin and co-workers (230) hypothesized that an increased cortisol to creatinine ratio in overnight urine samples reflected nocturnal hypoglycaemia in insulin-treated diabetics. Prasad et al (231) noted increased excretion of creatinine at weeks four and eight in alloxan diabetic rats. However other researchers have suggested that elevated serum

creatinine values in diabetics may be an artifact caused by ketoacidosis interference in the creatinine assay (232).

Low plasma creatinine has been reported in diabetes without ketonuria (233). Ditzel and co-workers (234) found mean values of plasma creatinine of 63 male and female diabetics without evidence of renal disease or ketoacidosis to be significantly lower than those in 123 healthy subjects. Glomerular filtration may be a factor in contradictory creatinine measurements reported in diabetics. The average glomerular filtration rate was significantly increased in insulin-treated diabetic subjects showing no signs of diabetic angiopathy (235). Because plasma creatinine is predominantly cleared by glomerular filtration, the reduced mean level of creatinine in plasma may be a result of the increased glomerular filtration rate and consequently of increased creatinine excretion found in some diabetics without angiopathy.

Steers treated with alloxan had a marked increase in serum creatinine concentrations; insulin treatment of the alloxanized animals decreased serum creatinine concentrations (236). Koszalska and Andrew (237) found that creatinuria induced by creatine loading can be prevented in the rat by the prior administration of insulin. Furthermore, the hypercreatinemia which was observed shortly after the intraperitoneal injection of a creatine load was suppressed by insulin, suggesting creatinuria prevention was related to insulin's effect on the blood creatine

concentration per se rather than to an effect on the kidneys.

Creatinine Measurement

Ketosis, specifically elevation of acetoacetate in serum may cause elevated creatinine levels by the picric acid method (238). Bilirubin also interfered with the measurement of plasma creatinine by the Jaffe reaction (239). Pyruvate, glucose, fructose, ascorbate and protein also interfered with the Jaffe reaction for creatinine by means of a side reaction with the alkaline picrate reagent (240). Major overestimation of creatinine was obtained in presence of acetoacetic acid, with relatively slight overestimation in the presence of acetone and glucose (241).

Picric acid methods for estimating creatinine greatly overestimated serum creatinine in mice. Both mouse and rat serum seem to have substances which gave nonspecific color and thus interfered with the analysis of creatinine by alkaline picrate methods. Thus HPLC is recommended to accurately measure rodent creatinine (242).

The conversion of creatine and creatine phosphate to creatinine in stored urine samples is affected by pH and temperature (241). The maximum rate of conversion of creatine to creatinine was found between pH 3.5 and 4.0. Thus at pH 3.5-4.0 and a temperature of 37 degrees C for 24 h, the creatinine content of urine rose by about 20%.

Longer periods of storage were associated with a greater increase in creatinine.

In summary, creatinine excretion is increased in stress (228), infection, fever, trauma (227) and in alloxan diabetes (231)(236). Increased glomerular filtration in diabetic subjects without angiopathy resulted in decreased plasma and increased urinary creatinine excretion (235). Elevated serum creatinine in alloxan treated animals (236) and creatinurea produced by intraperitoneal injections (237) were prevented by insulin treatment.

Factors Affecting 3-Hydroxy-3-Methyl Glutaryl Coenzyme A Reductase Activity

Sterol synthesis rate in the guinea pig was similar to those in the human. Depending on the time of day, the rate of sterol synthesis in the ileum was from 6 to 14 times that in the liver, while in the lung the rate was up to 3 times that of the liver (243). Sable-Amplis and Sicart (244) determined in the guinea pig that the cecum as well as the ileum of the guinea pig incorporated acetate into cholesterol and phospholipids at a rate greater than that of liver. Therefore, measurements of sterol synthesis in guinea pigs should focus as much on the intestine as on the liver.

In a chain of biochemical reactions any factor which modifies the rate-limiting step also modifies proportionately the rate of synthesis of the final product.

Under most physiologial conditions the rate limiting enzyme for cholesterol synthesis is 3-hydroxy-3-methyl glutaryl coenzyme A (HMG CoA) reductase (245,246). The reaction catalyzed by HMG CoA reductase (mevalonate: NADP oxidoreductase, EC 1.1.1.34) involves reductive deacylation of HMG CoA to mevalonate and requires 2 moles of NADPH (245).

A circadian rhythm of hepatic cholesterol synthesis was first demonstrated in mice by Kandutsch and Saucier (247). In rats hepatic cholesterol synthesis and HMG CoA reductase exhibit peak activities near midnight (245). However, peak activity of HMG CoA reductase was related to the time of feeding and did not require the onset of darkness (248). Sterol synthesis in guinea pig liver peaked at 3 h after the onset of the dark period; but variation between animals was high and the rate did not differ significantly with the time of day (243). The differences in the rat and guinea pig in the diurnal patterns of hepatic and intestinal sterologenesis may thus be explained by the marked difference in the feeding patterns of the two species. The rat has only one peak of eating activity during a 24 hr cycle and consumes at least 70% of its total daily intake during the dark period. The guinea pig, on the other hand, has three periods of increased eating activity during a 24 hr cycle with similar total feed consumption in the light and dark periods.

Diets high in carbohydrate and low in fat decreased the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (249). The activity of HMG CoA reductase was regulated by the degree of unsaturation of dietary fats; the more unsaturated the fatty acids, the lower the activity (250). HMG CoA reductase was lower in rats fed a semipurified diet containing sucrose, casein, and cellulose than in those fed a stock diet (251). Citrus pectin added to the semipurified diet produced HMG CoA levels equal to the stock diet (251). Fiber was hypothesized to be responsible for binding bile acids and increasing the turnover rate of cholesterol (251).

Whether bile acids themselves or the enterohepatic circulation of endogenous cholesterol play a direct role on hepatic cholesterol synthesis is questioned. When bile salts were infused into the portal vein, cholesterol synthesis was not inhibited (252). Harry et al (253) produced biliary obstruction in rats by surgical ligation. The biliary obstruction increased hepatic cholesterol to levels greater than those found at the peak of normal diurnal variation. In another study, bile acid content of the liver was increased by biliary diversion which increased sterol synthesis 2.5-fold (254). Increased sterol synthesis in rats with biliary diversion was prevented by the infusion of cholesterol in the form of chylomicrons. Weis and Dietschy (254) suggested there was no relationship between tissue content of bile acid and hepatic cholesterol synthesis. However, when Shefer et al (255) administered

bile acids at 1% of the diet, they found HMG CoA reductase activity was reduced.

Higgins (256) stated HMG CoA reductase activity was regulated by at least two mechanisms: the concentration of enzyme protein determined by modulation of its synthetic rate, and a more direct modulation of specific activity. These mechanisms were affected by cells' environment. Rao and co-workers (257) demonstrated that the depressed activity of hepatic HMG CoA reductase in starved or cholesterol-fed rats was stimulated by administering small quantities of ATP. Edwards (258) noted that catecholamines had a stimulatory effect on HMG CoA reductase activity which did not involve elevated concentrations of cyclic AMP as an intermediary.

Functioning adrenal glands and cortisol may be necessary for maximal activity of HMG CoA reductase. Konstantinos and colleagues (259) found that the diurnal rhythm was maintained for HMG CoA after adrenalectomy, but the amplitude of variation for the activity of the enzyme was greatly decreased. A single injection of cortisol administered to adrenalectomized rats 3 h before the expected maximum in enzyme activity resulted in a two-fold increase in enzyme activity. Other compounds including glucagon, dibutyryl cyclic AMP, actinomycin D and hydrocortisone suppress HMG CoA reductase activity in rats.

Activity of HMG CoA reductase was stimulated by thyroid hormone given in vivo to hypothyroid animals (260).

Fletcher and Myant (261) investigated the synthesis of cholesterol after destruction of the thyroid or injections of thyroxine. Thyroidectomy in guinea pigs depressed the rate of synthesis of cholesterol. Thyroxine stimulated cholesterol synthesis at low concentrations and inhibited at high concentration.

When normal or hypophysectomized rats fed a diet containing mevinolin and colestipol (a potent inhibitor of HMG CoA reductase and a bile acid sequesterant, respectively which act synergistically to stimulate the levels of reductase mRNA protein) were switched to a normal chow diet, HMG CoA reductase mRNA fell rapidly (262). In normal rats reductase activity and immunoreactive protein fell in parallel with the mRNA after a 90 min lag period. In hypophysectomized rats, reductase activity and protein required 10 and 18 hrs respectively to fall to 50% of their original levels. Administration of thyroid hormones to hypophysectomized rats resulted in a stabilization of reductase mRNA suggesting that the increased mRNA levels were due in part to a posttranscriptional regulatory effect of thyroid hormones (262).

Stimulation of reductase activity in hypophysectomized diabetic rats requires the mediation of both insulin and L-triiodothyronine (263). Neither hormone alone was effective. The rapid stimulation of reductase activity by insulin and the delayed stimulation elicited by Ltriiodothyronine are both inhibited by either glucagon or

hydrocortisone (263). Sample and Ness suggested that insulin acted by increasing the portion of HMG CoA reductase in its active free sulfhydryl form (264).

In streptozotocin induced diabetic rats, the major site of cholesterol synthesis was shifted from the liver to the small intestine. After one week, total reductase activity in small intestine was two and one-half times normal, whereas activity in liver remained low (265). Chronic insulin therapy markedly stimulated hepatic HMG CoA reductase activity in diabetic rats but did not increase gut enzyme activity (266).

Low hepatic HMG CoA reductase activity in alloxan diabetic rats was restored to normal by insulin treatment (267). Lakshmanan et al (268) reported a two to seven-fold increase of HMG CoA reductase activity and conversion of acetate to cholesterol after subcutaneous administration of insulin in normal or streptozotocin-treated diabetic animals. However, reductase activity was not changed when glucagon was administered in conjunction with insulin. Nepokroeff and co-workers (269) also reported stimulation of HMG CoA reductase activity to normal levels within two hours in streptozotocin-induced diabetic rats.

Phosphorylation/Dephosphorylation Control of HMG CoA

Beg and co-workers (270) in an in vitro experiment established that the reversible inactivation of HMG CoA reductase by ATP/magnesium was due to a covalent

phosphorylation-dephosphorylation reaction. Beg and colleagues (271) subsequently presented evidence that hepatic HMG CoA reductase undergoes covalent phosphorylation in vivo as well as in vitro. Ingebritsen et al (272) found added insulin enhanced and glucagon depressed cholesterol synthesis. Because reductase is active in the dephosphorylated state, the data indicated insulin signals net dephosphorylation, while glucagon brings about net phosphorylation.

Within 20 minutes of intravenous infusion of insulin into diabetic rats, HMG CoA reductase was totally dephosphorylated. Four hours of intravenous infusion of insulin into diabetic rats produced a five to six fold increase in total enzyme activity (273). Intraperitoneal injection of glucose (to raise serum insulin) into rats resulted in a transient (30 min) increase in the activity of HMG CoA (261). Conversely, intravenous injection of guinea pig anti-insulin serum depressed HMG CoA activity within 20 min. Intravenous injection of glucagon and adrenaline into normal rats did not affect the degree of phosphorylation of the HMG CoA reductase despite an increase in hepatic cyclic AMP concentration induced by glucagon and adrenaline treatment. However, when insulin secretion was inhibited, either by the induction of streptozotocin diabetes or by simultaneous infusion of somatostatin, glucagon treatment depressed the activity of HMG CoA reductase. Easom and Zammit (274) concluded that insulin had a dominant role in

regulating the phosphorylation state of hepatic HMG CoA reductase.

Scallen and Sanghvi (275) proposed phosphorylation and dephosphorylation were involved in the control of three key enzymes of cholesterol regulation. If cholesterol enters the liver cell, HMG CoA reductase would be inhibited by phosphorylation and biosynthesis of cholesterol would be reduced; however, reactions utilizing cholesterol would be activated due to the phosphorylation of acyl-CoA cholesterol acyltransferase (ACATase) and cholesterol 7 alpha-hydroxylase providing an elegant short-term mechanism for the homeostatsis of intracellular unesterified cholesterol.

Some researchers do not agree that short-term physiological changes in HMG CoA reductase are by phosphorylation. Dugan and co-workers (276) and Kleinsek et al (277) performed immunotitrations of rat liver HMG CoA reductase. They concluded that short-term physiological changes in HMG CoA reductase activity were created by a change in the quantity of enzyme due to conversion of enzyme to immunounreactive products and not by reversible phosphorylation of pre-existing enzyme. Kleinsek and co-workers (277) also reached a similar conclusion using immunotitrations of microsomal HMG CoA reductase.

Vitamin C Effects on HMG CoA Reductase

Activity of HMG CoA reductase was suppressed in human fibroblasts cultured in the presence of serum (278). Brown,

Dana, and Goldstein (279) indicated that apolipoprotein B, which is a component of low density lipoproteins (LDL) and very low densitiy lipoproteins (VLDL) is specifically involved in the suppression of the enzyme by serum. Binding of low density lipoproteins to membrane receptor sites of cultured fibroblasts resulted in the delivery of cholesterol to the cell in a form which decreased HMG CoA reductase activity. However, cultured cells from subjects with a homozygous form of familial hypercholesterolemia lacked the high affinity binding process and were resistant to suppression of HMG CoA reductase by low density lipoproteins (280).

Ginter and Juvcovicova (281) reported that chronic vitamin C deficiency lowered fractional catabolic rate of low-density lipoproteins in guinea pigs, decreased production of LDL receptors on the surface of liver cells, and prolonged half-life of plasma LDL. Based on these results, effects of vitamin C deficiency on HMG CoA reductase might be secondary to effects of the deficiency on LDL or LDL receptors.

The active (dephosphorylated) form of hepatic HMG CoA reductase was significantly reduced in guinea pigs fed inadequate or excessive dietary ascorbate (193). Greene and co-workers (282) found similar effects with hepatic microsomal incubations using deficient, physiologic, and supraphysiologic concentrations of sodium ascorbate. Thus,

dietary extremes of vitamin C may depress reductase activity.

Mineral Effects on HMG CoA Reductase

Information about mineral effects on HMG CoA reductase and cholesterol synthesis is limited. Gebhard and co-workers (283) reported increased activity of HMG CoA reductase in ileal mucosal homogenates in zinc-deficient rats. Curran (79) demonstrated that chromium enhanced the synthesis of cholesterol and of fatty acids in rat liver. Cupo and Donaldson (80) reported that chromium (20 ug/g diet) increased ^{14}C-acetate incorporation into liver fatty acids of chicks. Xi and co-workers (81) reported increased cholesterol in guinea pigs fed 4 ug Cr as $CrCl_3$/g diet versus 2 ug/g. However, whether chromium affects the activity of HMG CoA reductase has not been determined.

In summary, the rate-limiting enzyme for cholesterol synthesis is 3-hydroxy-3-methylglutaryl coenzyme A reductase (245,246). Catecholamines (258), cortisol (259), thyroid (260) and insulin (267) increased HMG CoA reductase activity, whereas cholesterol infusion (254), glucagon (259), and hydrocortisone in the absence of insulin (259) depressed cholesterol synthesis. Chronic ascorbate deficiency lowered LDL receptor sites; high affinity binding of LDL to these sites is a mechanism whereby cholesterol decreased activity of HMG CoA reductase (281). Both ascorbic acid deficiency and excessive ascorbic acid

depressed the enzyme's activity (193). Insulin appears to have a dominant role in the regulation of the phosphorylation state of HMG CoA reductase (274). Although chromium enhanced the synthesis of cholesterol in rat liver slices (79) and reduced circulating cholesterol in other studies (284), effects of chromium on the activity of HMG CoA reductase have not been reported.

CHAPTER III

EFFECTS OF STARCH, SUCROSE, FRUCTOSE, AND GLUCOSE ON CHROMIUM ABSORPTION AND TISSUE CONCENTRATIONS IN OBESE AND LEAN MICE

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Shortened Title:

CARBOHYDRATE EFFECTS ON CHROMIUM IN MICE

ABSTRACT

Forty-eight male genetically obese (OB) mice (C57BL/6J-OB) and 48 lean male littermates were randomly assigned within main plots (OB or lean) to one of eight diets. Diets were low Cr (-Cr) or supplemented with 1 mg/kg Cr as $CrCl_3$ (+Cr). Starch (ST), sucrose (S), fructose (F) or glucose (G) composed 50% of the diet which met AIN recommendations except for Cr. Experimental diets and deionized water were available ad libitum for 26 d. Mice were fasted 10 h and were intubated 2 h before sacrifice with 15 uCi of $^{51}CrCl_3$ in a 25% carbohydrate solution (2 mg carbohydrate/g body wt) of either starch, sucrose, glucose, or fructose corresponding to the diet previously fed. ^{51}Cr concentrations were significantly higher in the blood,

liver, spleen, epididymal fat pad, testes and femur of animals given their carbohydrate load as starch than in animals fed sucrose, fructose, or glucose. Carbohydrate had a significant effect on Cr concentrations of testes, spleen, kidney and liver with values generally being higher with the starch diet. Cr supplementation increased bone and kidney Cr concentrations and heart and muscle glycogen. These data indicate that source of carbohydrate can alter Cr absorption and retention.

KEY WORDS: Chromium, $^{51}CrCl_3$, chromium absorption, carbohydrate, obese mice

Interactions between essential trace elements and dietary carbohydrates have not been investigated thoroughly despite the major contributions of carbohydrate to the diet. Recent reports of carbohydrate effects on mineral absorption and metabolism have involved copper (1-7) and iron (8). Diets high in sucrose or fructose promoted deficiency of copper (2-4,7). However, iron retention was lower in iron-deficient starch-fed rats than in iron-deficient rats fed simple sugars (8).

Schroeder, Mitchener and Nason (9) noted a difference in the utilization of Cr with sucrose versus starch diets. Staub, Reussner and Thiessen (10) also reported that serum cholesterol was lower in rats fed starch than in those fed sucrose. Diets containing 60% glucose, fructose, or sucrose promoted more rapid induction of diabetes in the C57BL/KsJ-

db/db genetically diabetic mouse than a diet containing 60% dextrin (11). In human beings, diets high in simple sugars increased urinary Cr excretion (12).

Decreased sensitivity of peripheral tissue to insulin was observed in chromium deficiency (13). Obese mice (C57BL/6J-ob/ob) and their lean littermates were used in the present study because obese mice exhibit insulin resistance (14) and a previous study has shown them to be sensitive to chromium depletion (15).

The study was designed to determine if tissue concentration of Cr would be altered by the type of dietary carbohydrate fed and if ^{51}Cr absorption from $^{51}CrCl_3$ differed when intubated concurrently with one of four sources of carbohydrate (starch, fructose, glucose, and sucrose). Effects of Cr depletion and of the obese gene on Cr absorption and metabolism also were tested.

MATERIALS AND METHODS

Animals and Diets

Forty-eight male genetically obese (OB) mice (C57BL/6J-OB) and forty-eight lean mice (Jackson Laboratories, Bar Harbor, ME) were obtained at 4-5 wks of age. They were housed in plastic cages with wood shavings as bedding. The wood shavings were analyzed and were found not to be a source of Cr. The diet, provided in ceramic cups, and deionized water, in glass or plastic bottles with glass

sipper tubes, were available ad libitum. For the first 2 wks, animals were fed the American Institute of Nutrition (AIN) diet for mice and rats (16) modified to be low in Cr (.061 mg/kg). This basal diet contained 20% casein, 0.3% DL-methionine, 15% cornstarch, 50% sucrose, 5% celufil, 5% corn oil, 1% AIN 76-A vitamin mix (17), 0.2% choline and 3.5% AIN 76 mineral mix without Cr (prepared in our laboratory).

After the adaptation period, animals were randomly assigned within main plots (OB or lean) to one of eight experimental diets for 26 days. The 50% sucrose in the AIN diet was replaced by starch, fructose or glucose to form the following diet groups: starch, sucrose (AIN), fructose, or glucose. Each diet was formulated to have either a low Cr content (-Cr) or had 1 mg/kg of Cr as $CrCl_3$ added (+Cr). The analyzed Cr content of the low Cr diets (in mg/kg) was starch (.071), sucrose (.061), fructose (.057), and glucose (.047). These dietary concentrations do deplete animals of Cr; however, ingredients and contamination are continuously monitored to produce lower Cr diets.

Necropsy

Prior to killing, animals were fasted for 10 h. Animals were intubated (0700-0900) with a 25% carbohydrate solution (2 mg/g body weight) of either starch, sucrose, fructose or glucose corresponding to the carbohydrate previously fed. Fifteen microcuries of $^{51}CrCl_3$ (New England Nuclear, Boston,

MA) in 50 uL dilute HCl (pH 2.45) was mixed with the carbohydrate load for each mouse immediately preceding intubation. Two hours after the carbohydrate load, urine was collected and mice were anesthetized by sodium pentobarbitol injection (1.5 mg for lean and 2.5 mg for obese) in 0.9% saline. Mice were exsanguinated by cardiac puncture using syringes containing 2 mg EDTA. Blood samples were held in ice, individually counted in a gamma counter, weighed and centrifuged. Plasma was frozen for subsequent analyses. Liver, heart, and muscle samples were solubilized in 30% KOH for glycogen analysis immediately after exsanguination. The remaining tissues were removed carefully to avoid Cr contamination and were either weighed into glass tubes for gamma counting or were individually wrapped and frozen for Cr analysis.

Analytical Methods

Glycogen was assayed spectrophotometrically using anthrone reagent (0.2 g anthrone in 100 mL of 95% H_2SO_4) (18). Plasma glucose was determined using the glucose oxidase method with a glucose analyzer (Beckman Instruments, Inc., Fullerton, CA) (19). Plasma cholesterol concentrations were measured enzymatically (Method #352, Sigma Chemical Co., St. Louis, MO) (20).

^{51}Cr in blood was counted immediately in a gamma counter. Other tissues were counted as soon as possible and corrected

for isotope decay. The total counts for each tissue are presented as a percent of the initial dose.

For dietary Cr analysis, samples of diets (200-250 mg) were weighed into acid-washed borosilicate glass tubes and dried for 24 h at 100°C. Samples then were ashed in a muffle furnace with no exposed metal heating elements (Lindberg, Watertown, WI) using a modification of the method of Hill and coworkers (21) with a dry ashing temperature of 450°C. (The ashing temperature of 375°C as specified in the method now is being used in our laboratory.) After ashing, samples were diluted with 1N HCl (G. Fredrick Smith, Columbus, OH) and analyzed at 357.9 nM using a Perkin Elmer Model 5000 atomic absorption spectrophotometer with graphite furnace and Zeeman background correction (Perkin Elmer Corp., Norwalk, CT).

Glass knives were used to cut tissues and to clean the femur and tibia for Cr analysis. Drying and ashing procedures for tissues and bone were identical to diet analysis.

Statistical Analyses

The Statistical Analysis System (SAS) was used to evaluate treatment effects. Data were analyzed as a split plot design with obesity as the main effect and carbohydrate source and chromium supplementation as subplot factors. Log transformations were performed for statistical analyses when data were not normally distributed (noted in the tables).

The generalized linear model (GLM) was used for analysis of variance and least squares means determination.

RESULTS

Lean mice weighed less than obese mice (Table 1). The liver and epididymal fat pad of obese mice were heavier than lean mice whereas spleen, testes, and femur of obese mice weighed less than lean mice. Obese and lean mice did not differ in heart and kidney weight. Final body weight was not significantly affected ($p>0.05$) by either carbohydrate type or Cr level. Spleen weight was higher in sucrose than starch, fructose, or glucose groups. Mice fed Cr supplemented diets had increased femur wet weight and percent water in the bone. Weights of other tissues were not affected by dietary carbohydrate or by Cr supplementation.

Muscle and cardiac glycogen were higher in Cr supplemented animals but were not affected by carbohydrate or the presence of the obesity gene (Table 2). Hepatic glycogen increased with Cr supplementation (32 mg/g vs 25 mg/g) in obese but not in lean animals. An interaction of genetics and carbohydrate source on hepatic moisture was detected. Hepatic water content was higher in lean than in obese mice; carbohydrate effects were noted only in lean mice. The only variable that significantly affected plasma glucose and cholesterol was obesity; these both were greater for obese than lean mice.

Two hours after the ^{51}Cr dose, more ^{51}Cr was found in blood of animals given their carbohydrate load as starch than as sucrose, fructose, or glucose (Table 3). To calculate ^{51}Cr in blood, total blood supply of the mouse was estimated to be 7% of body weight. These values reflect a relatively high absorption of ^{51}Cr because absorption of Cr from $CrCl_3$ usually is low.

Obese animals fed starch had more ^{51}Cr in their livers than did lean mice; this difference generally was proportional to the liver weights of the two types of animals. In the spleen and testes, radioactivity likewise was higher in animals fed starch than other carbohydrates tested. However, total uptake by spleen and testes was very low. In the epididymal fat pad, obese animals had more ^{51}Cr than lean animals and differences due to dietary carbohydrates paralleled those in other tissues. More ^{51}Cr was found in the femur of the groups fed starch compared to sucrose and fructose, but starch was not significantly higher than glucose.

^{51}Cr in the kidney was highly variable and was not significantly affected by obesity or treatment. An interaction between carbohydrate and obesity affected ^{51}Cr excretion in urine. Obese mice given a carbohydrate load of starch excreted significantly more ^{51}Cr in the urine than mice given loads of sucrose or fructose (Figure 1). Lean animals given fructose loads had more urinary ^{51}Cr than lean

mice fed sucrose or glucose. Lean animals given a fructose load excreted more ^{51}Cr in urine than did obese animals.

The obese gene affected Cr concentration in all tissues analyzed except kidney (Table 4). The Cr concentration in the testes, spleen, and bone of obese animals was significantly higher than lean animals despite the interaction noted between Cr and obesity in the spleen and bone (Figures 2 & 3). In contrast, lean mice had more Cr per gram liver than did obese mice.

Type of carbohydrate fed affected Cr concentration in all tissues analyzed except bone. In the testes, starch and sucrose groups had higher Cr levels than did fructose or glucose groups. In the kidney, starch-fed animals had higher Cr than sucrose and fructose but not glucose-fed animals. In both spleen and liver, starch feeding significantly increased hepatic Cr concentration over glucose, fructose, or sucrose feeding.

Supplementation with 1 mg/kg Cr significantly increased Cr concentration in the kidney and bone. In the spleen an interaction between Cr and obesity occurred. In obese animals Cr supplementation increased splenic Cr concentration (149 ng/g vs 113 ng/g) while in lean animals Cr supplementation had no effect on Cr concentrations in the spleen (79 ng/g for both groups).

DISCUSSION

Differences in body and tissue weights due to gene were similar to results from a previous study (22). Although significant, the effect of carbohydrate on spleen weight was very small. The higher percent water in the bone of Cr-fed mice suggests that the organic matrix may change with supplementation. Whether this difference reflects protein, collagen, or some other factor remains to be determined.

Both obese and lean mice had higher muscle and cardiac glycogen with Cr supplementation which confirmed results reported by Mertz (23). Cr supplementation was a significant factor in increasing the hepatic glycogen of obese mice but not of lean mice in this study. Obese mice have elevated circulating insulin concentrations and insulin resistance (14). Because Cr potentiates the action of insulin (13), its effects on hepatic glycogen synthesis may be greater in obese than in lean mice. Hepatic moisture was higher in lean than obese mice which may reflect less lipid accumulation. Obese mice characteristically display elevated lipogenesis (14), and elevated hepatic lipid has been observed in response to Cr depletion (15).

Plasma glucose was not significantly affected by either Cr or carbohydrate. Gastric emptying patterns vary for different sugars (24). Because glucose was measured at a single time period, the high variability of the values reflects differences in absorption patterns of the

carbohydrates as well as animal-to-animal variation. Plasma cholesterol was not affected by dietary variables but our 26-day feeding period may have been too short to affect cholesterol.

At 2 h, a load of 2 mg/g body weight of starch increased ^{51}Cr absorption/retention compared with loads of glucose, fructose and sucrose in all tissues analyzed except kidney. Previous data have shown very rapid transit of $^{51}CrCl_3$ through the gastrointestinal tract (25) and rapid peaks in ^{51}Cr in the blood (26). While a longer time period for Cr absorption and tissue equilibration might have proven revealing, some mice at 2 h already had detectable amounts of ^{51}Cr in their feces and a longer absorptive period would have created more potential for contamination of urine from ^{51}Cr in the feces. ^{51}Cr in muscle was not counted but also would contribute to the total.

The high ^{51}Cr absorption in our study may reflect the fasting state preceding the $^{51}CrCl_3$ and lack of other dietary components in the gut to bind Cr or to alter the acidity of the ^{51}Cr dose. Dosing the $^{51}CrCl_3$ with the carbohydrate load may have stimulated absorption. There also may be species differences in absorption/retention of Cr. Absorption of ^{51}Cr in both rats and guinea pigs at 3 h was considerably lower (25,27) than observed in the present study.

Feeding supplemental Cr for 26 days did not affect uptake of ^{51}Cr. Some other studies also have reported no effect of

Cr status on absorption of Cr (28). However, in guinea pigs which had been fed a Cr depletion diet for 22 weeks, ^{51}Cr was greater in blood and liver of the Cr depleted groups (27); and in a human study, urinary Cr excretion (and presumably absorption) was related to intake when Cr intake was low (29).

Chromium concentration after 26 days on the experimental diets was higher in the testes, spleen and bone and lower in the liver of obese mice compared to lean mice. In a previous study, obese and lean mice were fed diets containing 1 mg/kg Cr, no differences in tissue Cr concentrations were attributed to obesity (30); however, the levels of Cr reported were several-fold higher than in tissues we measured and differences in analytical techniques need evaluation. Tissue concentrations of zinc, copper, manganese and iron were reported to be lower in obese than in lean mice but tissue Cr was not analyzed (31).

Cr supplementation significantly increased Cr in the kidney and in the bone in this study. Bone previously has been shown to reflect Cr supplementation (15). The effect of Cr supplementation on Cr concentration of bone and spleen was greater in obese than in lean mice. A similar trend was observed in the kidney. Hepatic Cr was not increased with Cr supplementation which indicates that the liver is not a major storage site for Cr. Data from studies using ^{51}Cr also show more accumulation of the isotope in kidney and bone than in other tissues (32,33)

Cr concentrations generally were higher in tissues from animals consuming the starch diet than in tissues from those consuming diets containing simple carbohydrates; differences parallel data from the 2 h $^{51}CrCl_3$ dose. The starch diet was slightly higher in Cr than the other diets, but the sub plot means for tissue Cr concentration for the starch group appeared higher than the subplot means for Cr supplementation; hence, these differences should not have been created by small variations in the Cr concentrations in the unsupplemented diets.

A diet containing starch should slow digestion or the passage of intestinal contents through the gut compared with diets containing more simple sugars. Initially the starch would exert less osmotic potential and draw less water into the intestine. Presumably Cr travels very rapidly through the gastrointestinal tract, possibly with water (25). If this hypothesis is correct, dietary components which would increase water entry into the intestine might decrease Cr absorption. Conversely, components which slow passage through the gut may increase Cr absorption.

In diabetic mice, a much higher mortality rate was observed with diets containing fructose or sucrose than with dextrin diets (11). Rats made diabetic with streptozotocin and fed sucrose containing diets also showed more severe signs of the disease than rats fed starch (34). Differences in Cr action have been noted in rats fed sucrose and starch diets (9), and urinary Cr losses in humans are stimulated by

diets high in simple sugars (12). Data from our study indicate clearly that consumption of starch enhances absorption and retention of Cr in several tissues.

In conclusion, a carbohydrate load of starch facilitated ^{51}Cr uptake compared with carbohydrate loads of sucrose, fructose or glucose. Similarly, starch-fed animals had higher tissue Cr than did fructose, glucose, or sucrose-fed animals. Thus, type of dietary carbohydrate affects Cr absorption and retention. Effects of consumption of dietary carbohydrates on Cr status of humans and research animals warrants further investigation.

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TABLE 1

Body and tissue weights of obese and lean mice fed four carbohydrates with or without added chromium (Cr)[1,2]

	Body wt	Liver	Epididymal fat pad	Spleen	Testes	Heart	Kidney	Femur wet wt	Femur moisture
	g	*g*	*g*	*g*	*g*	*g*	*g*	*g*	%
Main plot means									
OB	44.6 ± 0.5^{A}	3.09 ± 0.06^{A}	2.88 ± 0.05^{A}	0.046 ± 0.002^{B}	0.176 ± 0.004^{B}	0.155 ± 0.004	0.33 ± 0.01	0.053 ± 0.002^{B}	30.4 ± 1.0
Lean	30.4 ± 0.5^{B}	1.24 ± 0.06^{B}	0.57 ± 0.05^{B}	0.062 ± 0.002^{A}	0.197 ± 0.004^{A}	0.158 ± 0.004	0.34 ± 0.01	0.075 ± 0.002^{A}	32.6 ± 1.0
Subplot means									
Starch	37.3 ± 0.7	2.13 ± 0.09	1.80 ± 0.07	0.052 ± 0.003^{b}	0.186 ± 0.005	0.149 ± 0.006	0.32 ± 0.01	0.065 ± 0.003	32.3 ± 1.3
Sucrose	38.2 ± 0.7	2.30 ± 0.09	1.70 ± 0.07	0.063 ± 0.003^{a}	0.188 ± 0.005	0.160 ± 0.006	0.34 ± 0.01	0.067 ± 0.003	32.7 ± 1.3
Fructose	36.3 ± 0.7	2.10 ± 0.09	1.62 ± 0.07	0.050 ± 0.003^{b}	0.179 ± 0.005	0.156 ± 0.006	0.34 ± 0.01	0.063 ± 0.003	30.6 ± 1.3
Glucose	38.1 ± 0.6	2.14 ± 0.08	1.79 ± 0.07	0.054 ± 0.002^{b}	0.192 ± 0.004	0.161 ± 0.006	0.34 ± 0.01	0.062 ± 0.002	30.5 ± 1.2
−Cr	37.4 ± 0.5	2.17 ± 0.06	1.72 ± 0.05	0.055 ± 0.002	0.187 ± 0.003	0.160 ± 0.004	0.33 ± 0.01	0.061 ± 0.002^{y}	29.5 ± 0.9^{y}
+Cr	37.6 ± 0.5	2.16 ± 0.06	1.73 ± 0.05	0.054 ± 0.002	0.186 ± 0.003	0.153 ± 0.004	0.34 ± 0.01	0.067 ± 0.002^{x}	33.6 ± 1.0^{x}
Source of variation					*P*-values				
GENE	< 0.0001	< 0.0001	< 0.0001	< 0.001	< 0.0005	0.67	0.44	< 0.0001	0.45
CHO	0.13	0.32	0.23	< 0.005	0.28	0.40	0.20	0.48	0.48
Cr	0.80	0.97	0.92	0.79	0.77	0.20	0.06	< 0.007	< 0.002
CHO × Cr	0.92	0.10	0.50	0.10	0.85	0.14	0.91	0.83	0.61
GENE × Cr	0.87	0.31	0.15	< 0.05	0.76	0.93	0.55	0.94	0.49
CHO × GENE	0.77	0.10	0.40	0.37	0.72	0.75	0.79	0.99	0.32
CHO × GENE × Cr	0.90	0.34	0.88	0.11	0.78	0.69	0.90	0.44	0.53

[1]Values are least squares means ± SEM (GENE, n = 40–48; CHO, n = 20–24; Cr, n = 40–48).

[2]Within main plot or subplot means, means not sharing a common superscript letter are significantly different ($P < 0.05$) Significant interactions requiring further interpretation are discussed in the text.

TABLE 2

Tissue glycogen, hepatic moisture and plasma glucose and cholesterol of obese and lean mice fed four carbohydrates with or without added chromium (Cr)[1,2]

	Muscle glycogen	Cardiac glycogen	Hepatic glycogen	Hepatic moisture	Plasma glucose[3]	Plasma cholesterol
	mg/g	*mg/g*	*mg/g*	%	*mg/dl*	*mg/dl*
Main plot means						
OB	2 11 ± 0.19	1.60 ± 0.11	28.4 ± 1 9^{A}	48.7 ± 0.4^{B}	264 ± 14^{A}	262 ± 10^{A}
Lean	2.11 ± 0.19	1.21 ± 0.11	19.5 ± 1.9^{B}	62.2 ± 0 4^{A}	210 ± 11^{B}	122 ± 9^{B}
Subplot means						
Starch	1 98 ± 0.27	1.52 ± 0.15	25.2 ± 2.6	55.8 ± 0.6	258 ± 22	211 ± 14
Sucrose	2 18 ± 0.26	1.32 ± 0.14	22.6 ± 2.6	55.7 ± 0.6	251 ± 16	202 ± 14
Fructose	1.97 ± 0.26	1.34 ± 0.14	22.9 ± 2.6	55.9 ± 0.6	206 ± 16	173 ± 14
Glucose	2.31 ± 0.24	1.44 ± 0.13	25.1 ± 2.4	54.2 ± 0.5	234 ± 14	185 ± 13
−Cr	1 83 ± 0 18^{y}	1.26 ± 0.10^{y}	23.0 ± 1.8	55.0 ± 0.4	242 ± 13	197 ± 9
+Cr	2.40 ± 0 19^{x}	1.55 ± 0.10^{x}	24.9 ± 1.9	55.8 ± 0 4	231 ± 11	189 ± 10
Source of variation			*P*-values			
GENE	0.99	0.10	< 0.0003	< 0.0001	< 0 002	< 0 0001
CHO	0.73	0.70	0.82	0.08	0.14	0 21
Cr	< 0.03	< 0.04	0.45	0.15	0 54	0.56
CHO × Cr	0.36	0.72	0 49	0 39	0 47	0.27
GENE × Cr	0.62	0.51	< 0.05	0.74	0.51	0 40
CHO × GENE	0.43	0.61	0.85	< 0.05	0.10	0.13
CHO × GENE × Cr	0.32	0.79	0.79	0.83	0.07	0.41

[1]Values are least squares means ± SEM (OB, n = 40–48; CHO, n = 20–24; Cr, n = 40–48).

[2]Within main plot or subplot means, means not sharing a common superscript letter are significantly different ($P < 0.05$) Significant interactions requiring further interpretation are discussed in the text.

[3]Animals were given a carbohydrate load (2 mg/g body wt) 2 h before killing.

TABLE 3

^{51}Chromium (Cr) in tissues and urine of obese and lean mice 2 h after intubation with specified carbohydrate (2 mg/g body wt) and $^{51}CrCl_3$[1,2]

	Blood	Liver	Spleen	Testes	Epididymal fat pad[3]	Kidney	Urine	Femur
				% of dose				
Main plot means								
OB	2.3 ± 0.23	0.46 ± 0.04[A]	0.014 ± 0.003	0.021 ± 0.001	0.153 ± 0.047[A]	0.29 ± 0.04	0.61 ± 0.09	0.49 ± 0.07
Lean	1.4 ± 0.24	0.22 ± 0.04[B]	0.019 ± 0.003	0.025 ± 0.001	0.034 ± 0.047[B]	0.14 ± 0.04	0.74 ± 0.10	0.40 ± 0.07
Subplot means								
Starch	2.8 ± 0.33[a]	0.49 ± 0.05[a]	0.028 ± 0.004[a]	0.032 ± 0.002[a]	0.232 ± 0.064[a]	0.27 ± 0.06	0.99 ± 0.12	0.67 ± 0.09[a]
Sucrose	1.4 ± 0.31[b]	0.28 ± 0.05[b]	0.013 ± 0.004[b]	0.018 ± 0.002[c]	0.045 ± 0.064[b]	0.21 ± 0.06	0.38 ± 0.13	0.32 ± 0.09[b]
Fructose	1.6 ± 0.31[b]	0.27 ± 0.05[b]	0.012 ± 0.004[b]	0.019 ± 0.002[bc]	0.040 ± 0.064[b]	0.18 ± 0.06	0.70 ± 0.12	0.36 ± 0.09[b]
Glucose	1.8 ± 0.30[b]	0.32 ± 0.05[b]	0.012 ± 0.004[b]	0.024 ± 0.002[b]	0.058 ± 0.059[b]	0.20 ± 0.06	0.63 ± 0.12	0.44 ± 0.09[ab]
−Cr	1.8 ± 0.22	0.33 ± 0.04	0.018 ± 0.003	0.023 ± 0.001	0.128 ± 0.044	0.22 ± 0.04	0.73 ± 0.09	0.49 ± 0.06
+Cr	2.0 ± 0.23	0.36 ± 0.04	0.015 ± 0.003	0.024 ± 0.001	0.059 ± 0.046	0.21 ± 0.04	0.62 ± 0.09	0.41 ± 0.07
Source of variation				*P-values*				
GENE	0.10	< 0.05	0.39	0.18	< 0.004	0.21	0.48	0.51
CHO	< 0.007	< 0.02	< 0.03	< 0.0001	< 0.0001	0.69	< 0.007	< 0.04
Cr	0.61	0.55	0.35	0.58	0.55	0.89	0.33	0.35
CHO × Cr	0.32	0.25	0.47	0.35	0.20	0.32	0.80	0.53
GENE × Cr	0.10	0.18	0.20	0.08	0.32	0.73	0.10	0.23
CHO × GENE	0.49	0.43	0.13	0.07	0.19	0.38	< 0.03	0.19
CHO × GENE × Cr	0.48	0.26	0.70	0.48	0.07	0.23	0.29	0.29

[1]Values are least squares means ± SEM (GENE, n = 40–48; CHO, n = 20–24; Cr, n = 40–48).

[2]Within main plot or subplot means, means not sharing a common superscript letter are significantly different ($P < 0.05$). Significant interactions requiring further interpretation are discussed in the text.

[3]Data for epididymal fat pad were not normally distributed; logrithmic transformations were performed prior to statistical analysis.

TABLE 4

Tissue chromium concentration (ng/g dry wt) of obese and lean mice fed four carbohydrates for 26 d with or without added chromium (Cr)[1,2]

	Testes	Kidney	Spleen	Liver	Bone[3]
Main plot means					
OB	74 ± 6^{A}	78 ± 6	135 ± 8^{A}	27 ± 3^{B}	42 ± 3^{A}
Lean	54 ± 6^{B}	77 ± 6	78 ± 8^{B}	55 ± 3^{A}	25 ± 3^{B}
Subplot means					
Starch	77 ± 8^{a}	95 ± 7^{a}	132 ± 11^{a}	53 ± 5^{a}	38 ± 4
Sucrose	76 ± 8^{a}	58 ± 8^{c}	103 ± 10^{b}	33 ± 5^{b}	26 ± 4
Fructose	52 ± 8^{b}	68 ± 8^{bc}	92 ± 10^{b}	40 ± 4^{b}	38 ± 4
Glucose	52 ± 7^{b}	83 ± 7^{ab}	101 ± 10^{b}	37 ± 4^{b}	33 ± 4
−Cr	62 ± 6	63 ± 5^{y}	98 ± 7	44 ± 3	25 ± 3^{y}
+Cr	66 ± 6	89 ± 5^{x}	116 ± 8	37 ± 3	42 ± 3^{x}
Source of variation			*P*-values		
GENE	< 0 03	0.74	< 0.02	< 0 001	< 0.0001
CHO	< 0.03	< 0.005	< 0.05	< 0 02	0.12
Cr	0.57	< 0 0005	0.07	0.12	< 0 0001
CHO × Cr	0.64	0.10	0.80	0 12	0.10
GENE × Cr	0 98	0.06	< 0.05	0 78	< 0.05
CHO × GENE	0.28	0.06	0.59	0 43	0.40
CHO × GENE × Cr	0.85	0.27	0.42	0.84	0.58

[1]Values are least squares means ± SEM (GENE, n = 40–48; CHO, n = 20–24; Cr, n = 40–48).

[2]Within main plot or subplot means, means not sharing a common superscript letter are significantly different ($P < 0.05$). Significant interactions requiring further interpretation are discussed in the text

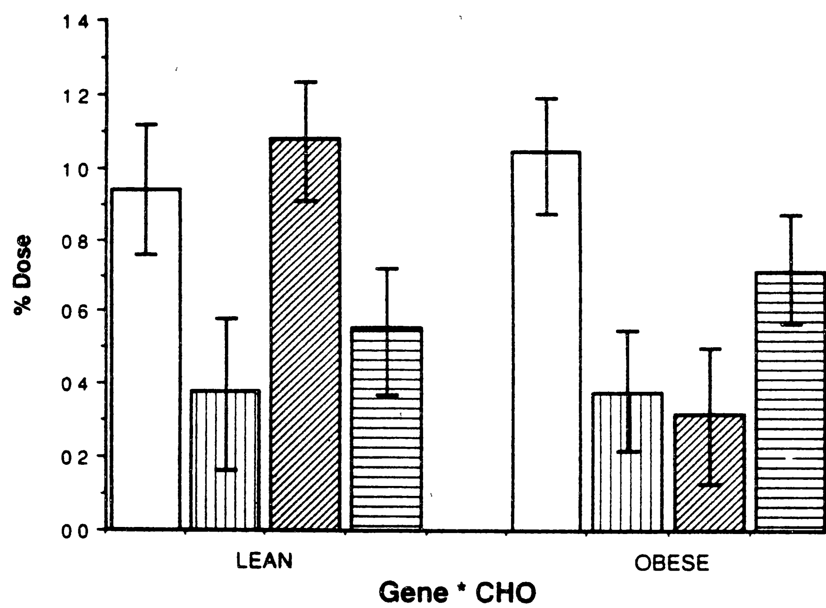
[3]Femur plus tibia.

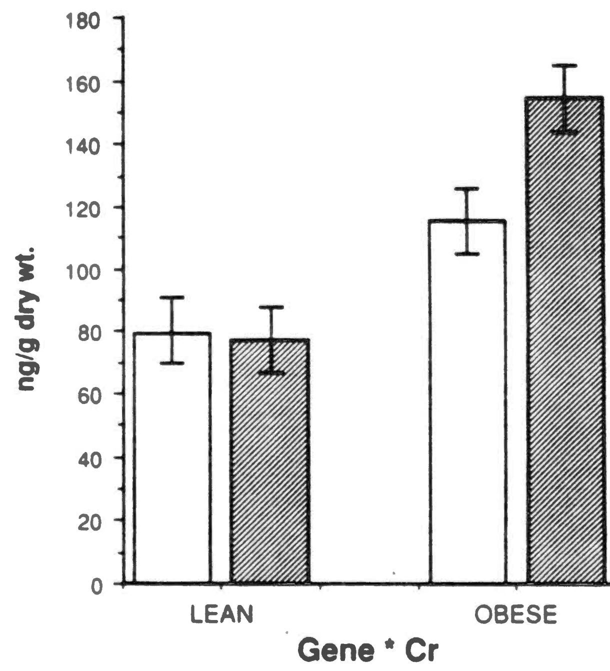
Legends for Figures

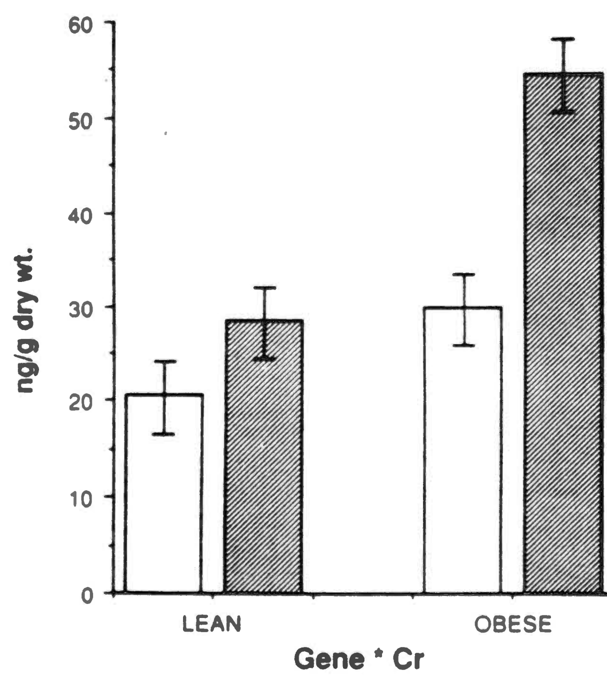
FIG. 1. Urinary ^{51}Cr excretion of lean and obese mice fed starch (open bars), sucrose (vertially hatched bars), fructose (diagonally hatched bars), or glucose (horizontally hatched bars).

FIG. 2. Splenic chromium of mice not supplemented (open bars) or supplemented (hatched bars) with chromium.

FIG. 3. Bone chromium of mice not supplemented (open bars) or supplemented (hatched bars) with chromium.


1 4
1 2
1 0
0 8
0 6
0 4
0 2
0 0
% Dose
LEAN
OBESE
Gene * CHO


180
160
140
120
100
80
60
40
20
0
ng/g dry wt.
LEAN
OBESE
Gene * Cr


60
50
40
30
20
10
0
ng/g dry wt.
LEAN
OBESE
Gene * Cr

CHAPTER IV

EFFECTS OF ANTACID OR ASCORBIC ACID ON TISSUE ACCUMULATION AND EXCRETION OF ^{51}CHROMIUM

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ABSTRACT

Absorption of trivalent chromium is low. Unbound chromium can form insoluble complexes in the gastrointestinal tract. In this study, effects of a calcium-based antacid, ascorbic acid or water on ^{51}chromium from chromium chloride were investigated. Rats were dosed with 1 mL of test substance (containing 150 mg calcium carbonate or 10 mg ascorbic acid) or 1 mL water. Twenty-four hours after dosing, ^{51}chromium in cumulative urine was lower ($p<0.02$) in the group dosed with calcium carbonate than in the groups dosed with ascorbic acid or water. Accumulation of ^{51}chromium in the kidney, testes, and spleen was lower ($p<0.05$) in rats dosed with antacid than in those dosed with ascorbic acid or water. These data confirm that absorption of chromium

chloride is low and suggest that antacids have a negative effect on chromium absorption from chromium chloride.

Key Words: Chromium, chromium chloride, antacid, ascorbic acid, calcium carbonate, ^{51}Cr

INTRODUCTION

Adequate trace mineral intakes cannot be assumed for all population subgroups. Recent estimates of chromium intakes in the United States were quite low with group means of 25-89 ug/day (1-4). Thus, many persons consume less than the current estimated safe and adequate intake of 50-200 ug chromium per day (5).

The elderly may be particularly at risk for adverse drug-nutrient interactions because of their chronic use of medications (6,7). In 1983 Americans spent 600 million dollars on antacids; sales of drugs for digestive diseases were increasing at a 10% rate (8). Gastrointestinal tract symptoms such as pain, flatulence and belching, as well as the gastric side effects of drugs including corticosteroids and aspirin, may promote antacid abuse in the elderly. In addition to use of antacids for gastrointestinal tract symptoms, an increasing number of women have responded to recommendations for increased calcium intakes (9) by taking calcium-based antacids.

Interactions of antacids and minerals have not been investigated thoroughly. Freeman and Ivy reported that

calcium carbonate and aluminum hydroxide reduced the retention of iron from iron sulfate fed to anemic rats (10). Magnesium trisilicate (35 g) given to iron deficient patients along with 5 mg of radiolabeled iron diminished iron retention in mildly iron deficient subjects (11). In normal, iron-replete subjects, 15 mL of either aluminum hydroxide, magnesium hydroxide, or magnesium carbonate-aluminum hydroxide antacid also reduced iron absorption from iron sulfate as measured by serum iron concentrations (12). Copper status also may be affected by antacids. A patient with decreased gastric emptying time who received a normal diet supplemented with antacids developed a severe copper deficiency (13).

Chromium can form insoluble complexes under alkaline conditions (14), raising the question of the impact of antacid use on chromium absorption. Several reports suggest that less than 2% of dietary chromium is absorbed (1,14,15), but information on drug-chromium interactions is not available. Because antacids are used widely by the elderly, their side effects on chromium status must be considered.

Several nutrient interactions affect trace mineral absorption. Although iron absorption is enhanced by ascorbic acid (16), effects of ascorbic acid on chromium absorption need investigation.

The measurement of chromium status in human beings is difficult; circulating chromium may not be in equilibrium with tissues store and its low concentrations require

sophisticated instruments for measurement (17,18). More sensitive evaluation of drug-nutrient interactions is possible through use of an isotope of chromium in an animal model. Thus, our study was designed to evaluate effects of an antacid, ascorbic acid or water on ^{51}chromium absorption by rats.

MATERIALS AND METHODS

Twenty-seven male Sprague Dawley rats with a mean weight of 354 g were assigned randomly to three treatment groups. They were fasted 12 hr and intubated with 1 mL of a suspension of calcium carbonate (150 mg), 1 mL of a 55 mM solution of ascorbic acid (10 mg), or 1 mL of distilled water. The pH of the distilled water, antacid, and ascorbic acid was 7.21, 8.21, and 2.88 respectively. Immediately after intubation, animals were dosed with 50 uL of ^{51}chromium chloride (20 uCi) in dilute hydrochloric acid (pH=1.89) by micropipette and placed in individual metabolic cages. Rats had access to water for the entire collection period and to a standard pelleted laboratory animal diet after 6 hrs.

Three and six hours after intubation of the test substance and dosing with ^{51}chromium chloride, blood samples were collected from the tail and the accumulated urine samples were removed from the metabolic cages. Urine was collected to avoid contamination with feces. At 24 hr, rats

were anesthetized with ether, exsanguinated by cardiac puncture, and tissues were collected.

^{51}Chromium in each tissue and in total urine was expressed as percent of the ^{51}chromium dose. Total ^{51}chromium in blood was estimated assuming that blood was equal to 7% of body weight. The Statistical Analysis System (SAS) was used for analysis of variance and calculation of least squares means (20).

RESULTS

A time by treatment interaction affected ^{51}chromium in blood. Blood collected at 3 hr from rats intubated with antacid contained less ^{51}chromium than following ascorbic acid or water dosage (Figure 1). At 24 hr the groups were not different ($p>0.05$) from each other.

In total kidney, testes and spleen obtained 24 hr post dosing less ($p<0.05$) ^{51}chromium was present in rats dosed with antacid compared with rats dosed with ascorbic acid or water (Table 1). In total liver, dosing with antacid tended to reduce ($p=0.06$) ^{51}chromium compared to dosing with ascorbic acid or water.

In the cumulative 24 hr urine collection, excretion of ^{51}chromium of rats intubated with ascorbic acid was higher ($p <0.02$) than from rats intubated with either water or antacid.

DISCUSSION

Twenty-four hours after dosing, total recovery of ^{51}chromium in urine plus in the tissues sampled accounted for only 0.13% for the group dosed with antacid, 0.20% for the group dosed with water, and 0.42% for the group dosed with ascorbic acid. Although these figures do not represent total chromium absorption, they do confirm that absorption of ingested chromium chloride is low and suggest that drugs may affect chromium uptake. In a study of iron absorption in humans, hydroxide-containing antacids did not decrease iron absorption, but two carbonates, sodium bicarbonate and calcium carbonate, reduced iron absorption (19). Although the hydroxide-containing antacid had greater acid-neutralizing capacity than doses of sodium bicarbonate or calcium carbonate, its effect on iron absorption was less (19). The carbonates may form insoluble complexes with soluble minerals in the gut. The effects of calcium carbonate on chromium may be similar depending not only upon pH but also upon formation of insoluble complexes and competition for absorption.

Compared with water or calcium carbonate, ascorbic acid increased cumulative urinary ^{51}chromium excretion at 24 hr after dosing. In blood at 3 hr, ^{51}chromium was higher ($p<0.05$) in rats dosed with either ascorbic acid or water than with calcium carbonate. ^{51}Chromium content of tissues was not increased by dosing with ascorbic acid compared to

water. However, dosing with antacid markedly reduced ^{51}chromium content in tissues compared to rats dosed with ascorbic acid or water.

On a body weight basis, the doses of ascorbic acid and antacid fed to the rats were 28 and 424 mg/kg. These dose levels were selected to investigate acute effects of the compounds on chromium absorption. At the dosage used, calcium carbonate reduced ^{51}chromium in blood at 3 hr, spleen, kidney, testes, and urine of rats intubated with antacid compared with those intubated with ascorbic acid or water. Further studies are needed to investigate chronic effects of moderate antacid or ascorbic acid use on chromium retention and excretion.

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Legend for Figure

FIG 1. ^{51}Chromium in blood of rats at 3 hr and 24 hr after dosing with ^{51}chromium chloride and water, antacid, or ascorbic acid.

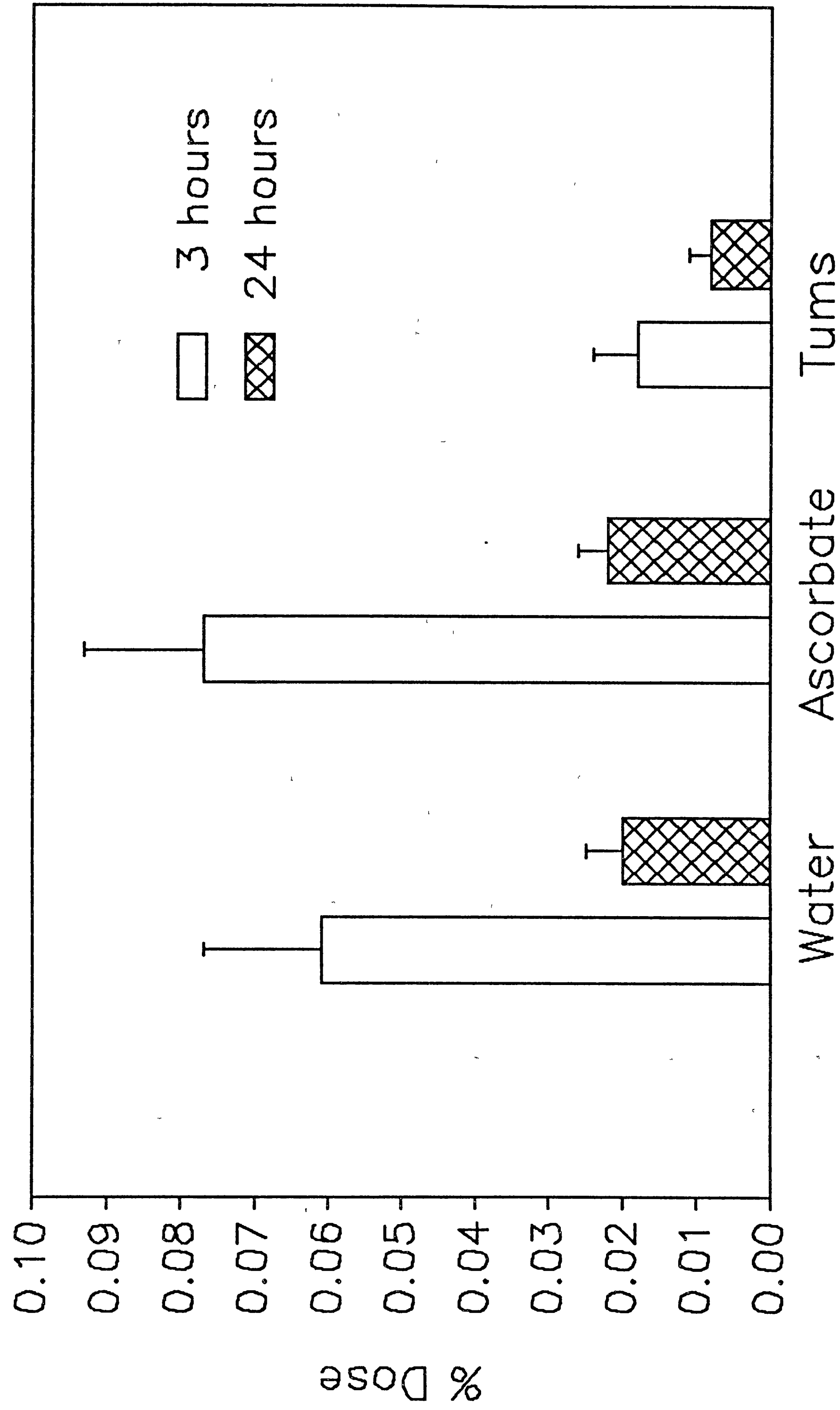
% Dose
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Water
Ascorbate
Tums
3 hours
24 hours

TABLE 1

^{51}Chromium in liver, kidney, testes, spleen and cumulative urine of rats 24 hr after dosing with ^{51}chromium chloride and water, antacid or ascorbic acid[1,2]

Treat-ment	Liver	Kidney	Testes	Spleen	Urine
			% Dose		
Water	0.005±0.001	0.007±0.002^{A}	0.0018±0.0004^{A}	0.00055±0.00010^{A}	0.165±0.066^{B}
Antacid	0.002±0.001	0.002±0.002^{B}	0.0005±0.0004^{B}	0.00007±0.00009^{B}	0.113±0.062^{B}
Ascorbic	0.006±0.001	0.007±0.001^{A}	0.0016±0.0003^{A}	0.00053±0.00009^{A}	0.386±0.055^{A}
Analysis of Variance					
P values	0.06	<0.04	<0.05	<0.003	<0.007

[1] Means are least squares means ± SEM (Water, n=7-8; antacid, n=8, ascorbic acid, n=10).
[2] Within a column, means not sharing a common superscript letter are significantly different (p<0.05).

CHAPTER V

RESEARCH NOTE

EFFECTS OF ASCORBIC ACID DEPLETION AND CHROMIUM STATUS ON RETENTION AND EXCRETION OF ^{51}CHROMIUM

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Ascorbic acid affects the absorption and retention of several minerals. Increased iron absorption from Fe(III)EDTA {2602} and decreased hepatic copper {2300} have been reported with dietary ascorbate supplementation. Likewise decreased uptake of selenium {2604} and nickel {2603} were observed with concurrent doses of ascorbate. Less than 2% of dietary inorganic chromium is absorbed {1054}. Chromium absorption may be impaired in chronic vitamin C deficiency. In scorbutic guinea pigs the total acidity of gastric juice was decreased {2411} which could reduce chromium absorption if olation and precipitation occurred in the more alkaline environment {1841}.

The effects of chromium status on chromium absorption and excretion have not been clarified {999}. Chromium absorption (estimated by urinary excretion) was inversely

related to dietary intake in human subjects {1141}. Hopkins and Schwarz {998} concluded that the amount of chromium absorbed from an oral dose of $^{51}CrCl_3$ was independent of chromium status of the rat.

Ascorbic acid deficiency affected the digestive and absorptive functions of the intestinal epithelium of guinea pigs {2601}. The intestinal uptake of glucose was augmented and the uptake of amino acids was appreciably impaired in chronic ascorbic acid deficiency {42}. A rapid uptake of glucose could also affect chromium retention. Glucose loading resulted in increased urinary excretion of chromium in human subjects not receiving chromium supplements {93}.

Hypoinsulinism and hyperglycemia have been observed in guinea pigs with ascorbate deficiency {2231}. Mertz proposed that chromium potentiates the action of insulin {135}. Insulin administration resulted in increased tissue retention of ^{51}chromium from $^{51}CrCl_3$ in diabetic rats {298}. Insulin deficiency and hyperglycemia impaired the uptake of ascorbic acid into cells {2206}. Diabetic subjects had lower blood vitamin C values than healthy subjects {183} and lower hepatic chromium {66}. Because of the reported derangement of glucose absorption and insulin in ascorbate deficient guinea pigs and of the debated role of previous dietary intake of chromium on chromium retention, we investigated the effects of chromium depletion and/or chronic ascorbic acid deficiency on tissue accumulation and

urinary excretion of ^{51}Cr from a dose of $^{51}CrCl_3$ followed by a glucose load.

Weanling male Hartley guinea pigs were randomly assigned to chromium and/or ascorbate depletion groups in a 2 x 2 factorial design. Animals were deprived to 0.5 mg ascorbate/day (-C) or supplemented with 10 mg/day (+C). Diets contained .2 ug/g Cr (-Cr) or were supplemented with 2 ug/g chromium as $CrCl_3$ (+Cr) and were fed for 24 weeks.

At the end of the experiment, the guinea pigs were fasted overnight. Three hours before necropsy, animals were dosed with 100 ul (80 uCi) of $^{51}CrCl_3$. One and one-half hours before necropsy a glucose load (50% solution) of 1 g/kg body weight was fed. Samples of blood, urine, liver, spleen and kidney were counted in a gamma counter. Total blood was estimated as 7% of body weight.

Means of tissue ascorbate in spleen, adrenal and liver of -C animals were 0.17, 0.38, and 0.15 mg/g respectively; means of +C animals were 0.51, 1.35, and 0.24 mg/g, respectively. With ascorbate depletion urinary excretion of ^{51}Cr was higher ($p<0.02$) than in +C animals. Tissue ^{51}Cr at 3 hours after the dose was not affected by ascorbate status. Thus ascorbate depletion did not inhibit Cr uptake as evidenced by tissue retention and urinary excretion of ^{51}chromium at 3 hr.

Animals fed -Cr diets had higher ^{51}Cr in blood ($p<0.05$) and liver ($p<0.04$) than those fed +Cr diets but urinary excretion of 51Cr was not affected by prior chromium intake.

An effect of dietary chromium on ^{51}Cr retention has not been seen in shorter studies {1157}; however, in this 24 week experiment, chromium deprivation enhanced the uptake of 51Cr from a dose of $^{51}CrCl_3$.

The increased urinary excretion of ^{51}Cr in ascorbate depleted animals given a glucose load has not been reported previously. Elevated urinary chromium values have been found in runners who exhibited elevated cortisol {3}, trauma patients {31}, and untreated diabetic humans {2242}. Brush border disaccharidases were stimulated in diabetes, a disorder resulting from lack of insulin {2605}, and cortisol elicited intestinal sucrase activity {2606}. Increased urinary excretion of ^{51}Cr may reflect a more rapid glucose uptake by intestinal epithelial and endocrine disturbances associated with the stress of ascorbate deficiency.

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TABLE 1

Comparison of ascorbic acid status and chromium supplementation on ^{51}chromium excreted in urine and retained in blood and tissues of guinea pigs supplemented with chromium and or ascorbate

Treat-ment	Blood	Liver	Spleen	Kidney	3 h Urine
			% Dose		
+Cr+C	0.089±0.016	0.012±0.003	0.0005±0.0002	0.0073±0.0016	0.0202±0.0064
+Cr-C	0.059±0.015	0.017±0.003	0.0006±0.0002	0.0053±0.0014	0.0525±0.0170
-Cr+C	0.041±0.015	0.008±0.003	0.0006±0.0002	0.0054±0.0015	0.0219±0.0078
-Cr-C	0.039±0.016	0.009±0.003	0.0004±0.0002	0.0059±0.0015	0.0859±0.0346
Analysis of Variance			p values		
Cr	<0.04	<0.04	0.88	0.68	0.34
C	0.33	0.24	0.68	0.61	0.02
Cr x C	0.36	0.54	0.50	0.41	0.39

CHAPTER VI

CHROMIUM AND CHRONIC ASCORBIC ACID DEPLETION EFFECTS ON ^{14}C RETENTION FROM ^{14}C-ASCORBATE, TISSUE ASCORBATE, AND AND TISSUE MINERALS IN GUINEA PIGS

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ABSTRACT

Chromium potentiates the effects of insulin; a role for insulin in ascorbic acid transport has been reported. Therefore, chromium and ascorbate depletion effects on ^{14}C distribution after a ^{14}C ascorbate dose were investigated in guinea pigs. For twenty weeks, forty weanling animals were fed either chromium deficient (-Cr) or chromium adequate casein-based diet (2 ug Cr from $CrCl_3$/g diet) and were deprived to 1 mg ascorbate/d (-C) or supplemented with 10 mg ascorbate/d (+C) for 20 weeks. Animals fed the chromium depleted diet exhibited decreased growth ($p<0.01$). However, creatinine and hydroxyproline concentrations in urine from fasted guinea pigs were unaffected by Cr or ascorbic acid status. Cr supplementation decreased plasma cortisol, brain ^{14}C and expired ^{14}C from a ^{14}C-ascorbate dose. Ascorbate supplementation increased hepatic ^{14}C, tissue ascorbate, and iron concentration in most analyzed tissues. Both

separately and interactively, ascorbate and chromium affected manganese concentration in several tissues. Cr supplementation increased Cr in liver, brain, adrenal, kidney, and spleen. The stress of confinement necessary to collect expired carbon dioxide may have affected distribution of ^{14}C from ^{14}C-ascorbate and tissue mineral concentrations.

KEY INDEXING WORDS: Chromium; guinea pigs; cortisol; iron; manganese; blood urea nitrogen; ^{14}C-ascorbate; ascorbic acid.

INTRODUCTION

Chromium has been shown to potentiate the effect of insulin on glucose entry into cells (1) and a role for insulin in ascorbic acid transport in human red blood cells has been reported by Mann and Newton (2). They found that glucose competitively inhibited transport of ascorbic acid and that hyperglycemia impaired intracellular ascorbic acid content. Kapeghian and Verlangieri (3) confirmed that ascorbic acid uptake by heart endothelial cells was compromised by decreased insulin and/or increased extracellular glucose levels. Diabetic rats have decreased tissue ascorbate concentrations (4) and diabetic humans have low tissue ascorbate concentrations (5). The major purpose of this study was to determine if chromium and ascorbic acid

status would affect tissue ascorbate concentration and ^{14}C-ascorbate retention and excretion in guinea pigs.

A close correlation between hydroxyproline excretion and growth rate has been determined (6). However, Bates (7) reported that hydroxyproline excretion was increased in C-deficient animals. Higher plasma corticosteroids were found in ascorbic acid deficient guinea pigs (8). Cortisol decreased total excretion of hydroxyproline in urine (9). Creatinine excretion increased with growth (10) and a high positive correlation between total body muscle mass and urinary creatinine excretion has been reported (11). Diabetic subjects had decreased plasma creatinine and increased creatinine excretion (12). Lack of chromium has been reported to affect growth in malnourished children (13) and in rats deprived of chromium (14). Therefore, in addition to body weight, other indicators of growth and protein utilization including creatinine, urinary hydroxyproline, and blood urea nitrogen were investigated in this study.

Absorption and metabolism of chromium are affected by interactions with other metals including zinc, iron, and manganese. Whole body contents of an oral dose of radioactive Cr were greater in zinc-deficient than zinc-supplemented rats (15). Chromium and iron may share a common gastrointestinal transport mechanism. Iron deficient animals absorbed more Cr from $CrCl_3$ than iron supplemented controls; iron depressed chromium binding to transferrin

(16). Transferrin formed a complex with manganese as well as chromium (17). Manganese (Mn) deficiency in the guinea pig induced a diabetes-mellitus like syndrome (18) which may reflect an interaction between Cr and Mn.

Major objectives of this study were to determine whether chromium depletion or chronic ascorbic acid deficiency and/or chromium and ascorbic acid supplementation in guinea pigs would affect tissue concentrations of certain trace minerals (manganese, iron, copper and zinc) and tissue ascorbate, blood concentrations of urea nitrogen and cortisol, hydroxyproline and creatinine excretion, and ^{14}C retention from a ^{14}C-ascorbate dose.

MATERIALS AND METHODS

Animals and Diets

Forty male Hartley guinea pigs (Sasco, Inc., Omaha, NE) with a mean weight of 165 g were assigned randomly to Cr and ascorbate treatment groups in a 2x2 factorial design. The basal diet contained <0.06 ug/g Cr (-Cr) or was supplemented with 2 ug/g Cr as $CrCl_3$ (+Cr) and were fed for 20 wk (Table 1). The mineral mix was prepared in a porcelain ball mill from minerals selected to be low in Cr. Diets were formulated after all individual components were assayed for chromium content. Copper, zinc, manganese, and iron concentrations of the -Cr and +Cr diets did not differ. To increase acceptability, the diet was mixed with deionized

water to form balls approximately 35 cm in diameter. Animals were dosed daily by micropipette with 1 mg ascorbate/d (-C) or 10 mg ascorbate/d (+C) dissolved in 100 ul deionized water.

Animals were housed in plexiglass cages with plastic grate floors. Animals had ad libitum access to food and deionized water from ceramic cups.

Sample Collection and Necropsy

Guinea pigs were weighed at 7 d intervals. Following a 12 h fast, urine was collected in a metabolic cage at week 8 and both fasted and nonfasted 12 h urine collections were obtained before the animals were sacrificed at week 20. Urine was measured and frozen for hydroxyproline, creatinine, and cortisol analyses.

Prior to necropsy, animals were weighed and fasted overnight. Six hours before sacrifice, each guinea pigs was dosed by micropipette with 90 ul (1.8 uCi) of L-[carboxyl-14C] ascorbate and placed in sealed metabolic cages. Expired carbon dioxide was trapped in three consecutive tubes of ethanolamine (40 ml per tube) and urine was collected. Vacuum pumps pulled expired air from the cages through the ethanolamine. Tissues, plasma and urine were frozen and processed later for scintillation counting of ^{14}C from the ^{14}C-ascorbate dose.

Animals were anesthetized in the metabolic cages with nitrogen gas and then exsanguinated by cardiac puncture.

Tissue samples for ascorbic acid analysis were frozen immediately in liquid nitrogen. Plasma was held on ice for ascorbate analysis or frozen for other analyses. The remaining tissues and bone (femur) were handled so as to avoid chromium contamination and were packaged individually in sealed plastic bags before freezing.

Biochemical Analyses

Hydroxyproline was determined in urine by the method of Bergman and Loxley (20) using internal standards. Urinary creatinine was determined by colorimetric analysis using a modification of the Jaffe reaction (alkaline picrate determination) (Method #555, Sigma Chemical Co, St. Louis, MO) (21). Urinary and plasma cortisol values were obtained by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). Plasma urea nitrogen was determined colorimetrically (Method #535, Sigma Chemical, St. Louis, MO) (23).

The tissues frozen in liquid nitrogen at necropsy were analyzed for ascorbic acid within 4 h of necropsy. These samples were ground in cold glass homogenizers and deproteinized in 5 ml of cold 10% trichloroacetic acid (TCA). Plasma was deproteinized using 2 volumes of cold 10% TCA. Ascorbate was analyzed using the ferrozine method (22). Interference by metals was minimized by using acid washed glassware and deionized water for all reagents.

Treatment of samples for scintillation counting of ^{14}C involved solubilization of 60-100 mg of tissue in 1 ml of 1N sodium hydroxide and incubation at 80° C for 15 min. The samples then were decolorized by adding 200 ul of hydrogen peroxide and incubating at 80° C for 30 min. The samples were mixed with 100 ul of 80% acetic acid, followed by 15 ml Insta-gel (Packard Inc., Downers Grove, IL) and were counted. Plasma (200 ul) was treated similarly before counting. Ethanolamine (0.2 ml) was added to 20 ml Insta-Gel and urine (0.6 ml) was added to 5.0 ml Insta-Gel. All samples were counted for 5 min in a Tri-Carb 1900CA Liquid Scintillation Analyzer (Packard, Laguna Hills, CA).

Samples of diet for chromium analysis (200-250 mg wet weight) were weighed into acid-washed borosilicate glass tubes and dried for 24 h at 100 degrees C. Samples then were ashed in a muffle furnace with no exposed metal heating elements (Lindberg, General Signal, Watertown, WI) using a modification of the method of Hill and co-workers (24) with a dry ashing temperature of 375° C. After ashing, these samples were diluted with 0.5% nitric acid (double distilled, G. Fredrick Smith, Columbus, OH) and analyzed at 357.9 nm using a Perkin-Elmer Model 5100 atomic absorption spectrophometer with a HGA 600 graphite furnace and Zeeman background correction (Perkin Elmer, Norwalk, CT).

Drying and ashing procedures for tissues and bone were identical to those for diet. Glass knives were used to cut tissue and clean bone before Cr analysis. Manganese and

copper tissue concentrations also were determined by graphite furnace atomic absorption spectroscopy (AAS); zinc and iron were determined by flame AAS.

Statistical Analysis

The Statistical Analysis System (SAS) was used to evaluate treatment effects. Data were analyzed as a 2x2 factorial design. The generalized linear model (GLM) was used for analysis of variance and least squares means determination.

RESULTS

Body and tissues weights of guinea pigs are shown in Table 2. Starting at week 13, animals fed the Cr depletion diet (-Cr) weighed significantly less than animals fed the supplemented diet with 2 ug/g Cr (+Cr). Ascorbate treatment did not affect body weight. Weights of the liver, kidney, testes, and epididymal fat pad were lower for animals fed the Cr depleted diet. Chromium treatment did not affect weights of brain, adrenal and spleen.

At week eight, the urine volume was higher for -Cr fed animals. Urine volumes were not significantly different for either the fasted or fed urine collections at week twenty (data not shown). Neither ascorbic acid or chromium intake affected urinary creatinine or hydroxyproline (Table 3) at week eight or week 20. Cortisol excretion in urine of

fasted animals at week 20 was not affected by Cr or ascorbic acid treatment.

Plasma cortisol concentration after animals had been restrained in metabolic cages for 6 h was higher ($p<0.05$) in -Cr animals than in +Cr animals. Ascorbic acid treatment did not affect plasma cortisol concentration. There was a significant interaction between Cr and ascorbic acid on blood urea nitrogen. In animals supplemented with 10 mg/day ascorbate, chromium supplementation increased blood urea nitrogen.

Tissues analyzed for ascorbic acid are shown in Table 4. Ascorbic acid supplementation of 10 mg/d increased ($p<0.0001$) ascorbate in all tissues. Ascorbic acid concentration in liver was 0.07 mg/g for those animals receiving 1 mg ascorbate per day versus 0.28 mg/g for those animals receiving 10 mg ascorbate per day. Plasma ascorbate was lower than tissues but was increased by the 10 mg ascorbic acid supplement. Cr treatment affected ascorbate concentration in the testes. The chromium-supplemented animals had lower ($p<0.03$) testes ascorbate concentration than animals maintained on chromium deficient diets.

The primary route of excretion of ascorbate in the guinea pig is via expired carbon dioxide. As shown in Table 5, the percentage of the ^{14}C-ascorbate dose that was expired as carbon dioxide in the 6 h collection period was greater ($p<0.04$) in guinea pigs fed the low chromium diet than in guinea pigs fed the chromium supplemented diet. The low Cr

diet also increased ($p<0.001$) retention of ^{14}C in the brain. However, retention of the ascorbate dose by the brain (0.04 to 0.06%) was very low compared to the liver which had a retention of 1.6 to 2.4% of the dose. The animals supplemented with 10 mg ascorbate retained a greater percentage of the ^{14}C ascorbate dose in the liver than animals given 1 mg ascorbate per day ($p<0.03$).

There was an interaction between intakes of Cr and ascorbic acid on ^{14}C retention in certain tissues. In the C depleted animals Cr decreased retention of ^{14}C from the ^{14}C-ascorbate dose but increased the fraction found in plasma and recovered in urine. Urine of all groups contained less than 4% of the dose at 6 hr. When blood volume was estimated at 7% of animal weight, plasma contained less than 2% of the ^{14}C dose at 6 hr.

Manganese in liver was higher in +Cr animals (Table 6). In the kidney, manganese was higher in -Cr animals. Splenic manganese tended to be increased ($p<0.06$) with Cr supplementation. Ascorbic acid supplementation increased manganese concentration in the adrenal, and testes but decreased manganese in the spleen. Interactions between chromium and ascorbic acid were found for manganese concentrations in bone and brain. In ascorbic acid depleted animals, Cr supplementation tended to increase Mn in bone but decreased Mn content of brain.

Zinc tissue levels were not affected by Cr or ascorbate treatment (data not shown). Copper concentration was

decreased ($p<0.007$) by ascorbate supplementation in kidney only. The kidney of animals receiving 10 mg ascorbate per day had 16 ug copper/g dry wt, whereas animals receiving 1 mg ascorbate had 19 ug copper/g dry wt in the kidney. Tissue copper concentrations were not affected by Cr treatment. Ascorbate supplementation increased iron concentrations in the liver, brain, testes, kidney and spleen (Table 7), but no differences were seen in tissue iron concentration as a result of chromium treatment.

Chromium supplementation increased Cr concentration in liver, brain, adrenal, kidney, and spleen (Table 8). In contrast, chromium in the testes was higher for Cr depleted than Cr supplemented animals. The Cr concentration in these tissues was not affected by ascorbate treatment. However, bone had a lower Cr concentration when 10 mg than when 1 mg ascorbate/d was fed.

DISCUSSION

The lower body weight of -Cr guinea pigs supports findings in the rat, mouse, and turkey and emphasize the essentiality of Cr for animal growth (25). The larger liver, kidney, testes, and epididymal fat pad weights of Cr supplemented animals suggest that Cr is involved in both protein synthesis (26) and glucose incorporation into fatty acids (27). However, no detrimental effect of -Cr diets on growth was evidenced by hydroxyproline and creatinine excretion. In animals supplemented with 10 mg ascorbate/d,

chromium supplementation decreased plasma urea nitrogen. Plasma cortisol measured in guinea pigs after being restrained for 6 h in metabolic cages was higher for -Cr animals. Elevated cortisol in the chromium deprived group may be induced by stress. The fasted urinary values for cortisol obtained at week 20 prior to immobilization in the metabolic cage showed no significant differences for Cr or ascorbate treatment. Also, adrenal weights were not altered by Cr treatment. Plasma cortisol values of guinea pigs receiving all treatments were double values reported by Kipp and Rivers (28) for non-stressed guinea pigs but similar to or below cortisol concentrations of guinea pigs which had been injected with adrencorticotrophin (ACTH).

The higher tissue ascorbate concentrations with ascorbate supplementation were expected (29). Plasma ascorbate concentrations were similar to values reported for guinea pigs 4 h after ACTH (30). Their plasma concentrations were elevated only slightly with their ACTH treatment (30). Tissue ascorbate concentrations in our guinea pigs also were similar to values of animals treated with ACTH at 4 h. Kipp and Rivers did not find tissue values of guinea pigs fed 500 mg ascorbate/kg diet to be changed by ACTH injection. Adrenal ascorbate values in guinea pigs treated with ACTH were higher at 4 hours (0.39 mg/g) than untreated guinea pigs. Adrenal ascorbate values of guinea pigs in this study (0.45 to 2.1 mg/g) were higher than in guinea pigs treated with ACTH. However, guinea pigs

subjected to the stress of swimming had decreased ascorbate in the adrenal glands, spleen and brain (31). Testes had lower ascorbate when treated with +Cr; this seems to parallel the ^{14}C ascorbate data.

Considering the indication of stress (cortisol values), the ^{14}C ascorbate data probably reflects the disposition of ascorbate under stress rather than under physiological distribution. In rats, acute immobilization stress increased serum ascorbic acid levels (32). Distribution of a dose of newly absorbed ascorbic acid in these guinea pigs may be altered by stress. ACTH treated animals retained 0.01% of an ascorbate dose in plasma at 4 h (30); by comparison, our guinea pigs retained nearly 2% of the dose in the plasma. The percent of dose retained in the tissues of immobilized guinea pigs was less than for non-stressed animals (30).

The ^{14}C in expired carbon dioxide by 6 hours was at the lower end of the expected range (5-24% of the dose)(33). The amount of ^{14}C in expired CO_2 was higher in -Cr treated guinea pigs. This indicates utilization or metabolism of the ^{14}C-ascorbate dose was increased by Cr deficiency. In other work, ketone bodies brought about a significantly higher destruction of ascorbic acid (34). Whether -Cr animals developed ketosis during immobilization stress was not determined. However, because the -Cr animals expired similar amounts of ^{14}C in carbon dioxide to that reported in untreated guinea pigs, some physiological condition existed

in +Cr animals that increased retention or conservation of the ascorbate during immobilization stress. Whether retention would be increased similarly by Cr in non-immobilized animals was not confirmed. Chromium at 5 ug/g diet increased the concentration of ascorbic acid in liver and adrenals of rats (35).

Effects of Cr on manganese concentrations have not been reported previously. Liver manganese was higher ($p<0.05$) and kidney manganese was lower ($p<0.02$) with Cr supplementation. Splenic manganese tended to be higher ($p<0.06$) in +Cr animals. Tissue manganese also reflected ascorbate supplementation; 10 mg ascorbate/d increased Mn in adrenals ($p<0.05$) and testes ($p<0.01$) but decreased Mn in spleen ($p<0.05$). The interaction between Cr and ascorbate on Mn in bone and brain indicate further that both Cr and ascorbate status may affect manganese retention.

In male Hartley guinea pigs, supplementation with ascorbic acid (25 mg/100 g bw per day) decreased liver copper by 200 to 300% (19). In men, ascorbic acid (500 mg) also was antagonistic to copper status (36). In our study 10 mg ascorbate/d reduced kidney copper concentration.

Hepatic iron concentration was increased in guinea pigs when a high level, 25 mg ascorbate/100 g bw, was fed (19, 37). In our study providing 10 mg ascorbate/d, iron concentration was increased in four of the seven tissues analyzed. These results indicate that effects of ascorbate supplementation on mineral status in animals and man should

be re-evaluated considering the possibility of hemochromatosis. Increased iron in tissues also may interfere with absorption and transport of manganese and other minerals (17). An increased iron concentration in tissues was associated with increased utilization of ascorbate (38).

Chromium concentrations in the liver, brain, kidney, and spleen although variable, increased with Cr supplementation. Concentrations in this study might be altered due to the stress of immobilization. In contrast to other tissues, chromium in testes was higher ($p<0.04$) in -Cr than in +Cr animals. Fertility and sperm count have been decreased in animals fed chromium deficient diets (39), but no information has been reported previously on Cr concentration of the testes in Cr deficiency. Bone chromium was decreased by ascorbate supplementation. As bone represents a Cr storage site, size of the Cr pool available for mobilization during stress may be decreased by ascorbate deficiency.

Stress affects chromium metabolism (40). Trauma, infection, surgery, intense heat or cold, all elevated secretion of hormones and either directly or indirectly affected chromium metabolism (41). After trauma in humans, plasma cortisol was elevated (42). The animals that were fed -Cr diets in this study exhibited increased cortisol after confinement stress. Urinary chromium excretion and plasma cortisol increased in runners (43); urinary chromium excretion was 10 times greater by traumatized patients 42 h

following hospital admission than by normal, healthy subjects. Whether the tissues give up their chromium or take up the circulating Cr in response to stress is not known. Pekarek and co-workers reported that subjects infected with sandfly fever had lower fasting serum chromium than healthy subjects (44), but their serum chromium concentrations are high by today's standards.

In summary, ascorbate and Cr status altered tissue ascorbate concentrations and distribution of ^{14}C from ^{14}C-ascorbate in confined animals. Adrenal ascorbate of all treatment groups (under immobilization stress) was higher than previously reported; ascorbate concentrations in other tissues were similar to previously reported values for guinea pigs. Testes had higher ascorbate and Cr concentrations in animals given the low Cr diets; however, some of this increase may be due to the 26% decrease in testes weight of guinea pigs on this diet. Expired ^{14}C was lower in +Cr animals indicating that some mechanism may exist to protect the ascorbate from oxidative destruction in +Cr animals. Manganese concentration of liver was increased and kidney was decreased by Cr supplementation; Mn concentrations in brain, adrenal and testes increased but spleen decreased with ascorbate supplementation and interactions of Cr and ascorbate on bone and brain Mn were detected. These interactions require further investigation to determine whether Cr, ascorbic acid, and Mn in combination may interact to affect lipid metabolism and

glucose utilization. The immobilization stress of our guinea pigs (necessary to collect expired CO_2) raises the question of the role of stress in chromium metabolism and whether cortisol may change Cr distribution. The increased iron in several tissues, decreased copper in kidney and decreased bone Cr with 10 mg/d ascorbate supplementation are of concern. The effects of ascorbic acid supplementation on chromium and manganese status in stress need evaluation.

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TABLE 1

Composition of the Diet[1]

Component	g/kg
Casein	300
Arginine	3
Dextrose	379
Celufil[2]	150
Corn oil	70
Vitamin mix[3] (without ascorbic acid)	22
Potassium acetate	35
Mineral mix[4] (without chromium)	50

[1]By analysis the -Cr diet contained <50 ppb Cr and the +Cr diet contained 2 ppm.
[2]US Biochemical Corp.
[3]The vitamin mix contained per kg (in g except as noted) alpha-tocohperol 5.0; choline chloride 75.0; d-calcium pantothenate, 3.0; inositol, 5.0; menadione, 2.25; niacin, 4.5; paraaminobenzoic acid, 5.0; pyridoxine HCl, 1.0; riboflavin, 1.0; thiamine HCl, 1.0; vitamin A acetate, 900,000 units; calciferol (D_2) 100,000 units; biotin, 20 mg; folic acid, 90 mg; and vitamin B_{12}, 1.35 mg.
[4]The mineral mix contained (g/kg): $CaHPO_4$, 600.0; NaCl, 80.0; MgO, 100.0; $ZnCO_3$, 0.9; $MnCO_3$, 1.8; $CuCO_3 \cdot Cu(OH)_2$ H_2O, 0.25; KIO_3, 0.035; $NaSeO_3 \cdot 5H_2O$, 0.0044; and $FeSO_4$, 1.4235.

TABLE 2

Body and tissue wet weights of guinea pigs fed diets with or without added Cr and given marginal ascorbate (1 mg/day) or supplemented with ascorbate (10 mg/day)[1]

	Body Wt.	Liver	Epididymal fat pad	Spleen	Testes	Heart	Kidney	Adrenal	Brain
	g	g	g	g	g	g	g	g	g
-Cr	635±18	21.07±0.88	4.72±0.35	0.99±0.06	1.03±0.09	1.97±0.07	4.77±0.19	0.39±0.03	3.71±0.06
+Cr	733±17	23.72±0.84	6.08±0.33	1.00±0.06	1.39±0.09	2.17±0.07	5.39±0.19	0.41±0.03	3.75±0.06
-C	702±18	24.45±0.90	5.82±0.36	1.07±0.07	1.28±0.09	2.03±0.07	5.33±0.19	0.39±0.03	3.75±0.06
+C	666±17	20.34±0.82	4.97±0.32	0.92±0.06	1.14±0.09	2.11±0.07	4.83±0.18	0.41±0.03	3.70±0.05
-Cr-C	653±26	22.81±1.34	5.20±0.53	1.06±0.10	1.17±0.13	2.01±0.11	4.71±0.28	0.40±0.04	3.70±0.08
-Cr+C	618±23	19.33±1.12	4.23±0.44	0.93±0.09	0.89±0.12	1.94±0.10	4.83±0.25	0.38±0.04	3.71±0.07
+Cr-C	752±25	26.08±1.19	6.44±0.47	1.08±0.09	1.38±0.13	2.06±0.10	5.95±0.26	0.38±0.04	3.79±0.08
+Cr+C	715±25	21.35±1.18	5.71±0.47	0.91±0.09	1.39±0.13	2.28±0.10	4.83±0.26	0.43±0.04	3.70±0.08
Source of Variation				P Values					
Cr	<0.01	<0.04	<0.01	0.99	<0.01	0.06	<0.03	0.71	0.62
C	0.16	<0.01	0.08	0.11	0.30	0.47	0.06	0.68	0.59
Cr x C	0.98	0.61	0.81	0.83	0.24	0.18	<0.03	0.35	0.51

[1]Values are least squares means ± SEM (Cr, n = 18-20; C, n = 19).

TABLE 3

Creatinine and hydroxyproline in fed and fasted urine, plasma and urinary cortisol and blood urea nitrogen of guinea pigs with or without added Cr and given marginal ascorbate (1 mg/day) or supplemented with ascorbate (10 mg/day)[1]

	Urinary Creatinine			Urinary Hydroxyproline			Cortisol		Plasma Urea Nitrogen
	Fasted (week 8)	Fasted (week 20)	Fed (week 20)	Fasted (week 8)	Fasted (week 20)	Fed (week 20)	Plasma (week 20)	Fasted urine (week 20)	(week 20)
		mg/ml			ug/mL		ng/mL		mg/dl
-Cr	4.95±0.37	7.49±0.60	11.39±1.15	2247±470	751± 82	1070±105	910± 99	174±28	29±1
+Cr	5.05±0.37	7.67±0.60	12.32±1.14	2187±467	644± 77	939±104	625± 94	175±27	27±1
-C	5.15±0.38	7.97±0.62	12.16±1.18	2119±481	649± 85	880±107	705±101	201±28	30±1
+C	4.85±0.36	7.20±0.59	11.55±1.11	2315±455	746± 73	1130±101	830± 93	148±27	27±1
-Cr-C	5.50±0.56	7.03±0.91	11.39±1.72	2670±700	631±129	910±156	844±152	168±41	29±2
-Cr+C	4.41±0.50	7.96±0.81	11.41±1.54	1822±626	871±100	1230±139	977±127	181±39	30±2
+Cr-C	4.82±0.52	8.90±0.85	12.94±1.62	1568±660	667±118	847±147	567±134	235±39	31±2
+Cr+C	5.29±0.52	6.44±0.58	11.69±1.62	2806±660	621±105	1029±147	683±134	116±39	24±2
Source of Variation				P Values					
Cr	0.85	0.84	0.58	0.93	0.35	0.38	<0.05	0.99	0.26
C	0.57	0.38	0.71	0.77	0.39	0.10	0.37	0.17	0.16
Cr x C	0.15	0.06	0.70	0.12	0.21	0.65	0.95	0.10	<0.05

[1]Values are least squares means ± SEM (Cr, n = 18-20; C, n = 19).

TABLE 4

Ascorbate concentrations of plasma and tissue (wet wt) in guinea pigs fed diets with or without added Cr and given marginal (1 mg/day) or adequate ascorbate (10 mg/day)[1]

	Plasma	Adrenal	Brain	Kidney	Liver	Spleen	Testes
	mg/ml	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g
-Cr	0.006±0.0006	1.31±0.12	0.35±0.02	0.13±0.008	0.17±0.01	0.40±0.03	0.40±0.03
+Cr	0.006±0.0005	1.18±0.12	0.33±0.02	0.11±0.008	0.17±0.01	0.40±0.03	0.32±0.03
-C	0.003±0.0006	0.48±0.12	0.13±0.01	0.05±0.008	0.07±0.01	0.13±0.03	0.13±0.03
+C	0.008±0.0005	2.01±0.12	0.57±0.01	0.18±0.008	0.28±0.01	0.67±0.03	0.58±0.03
-Cr-C	0.002±0.0008	0.51±0.18	0.13±0.03	0.05±0.010	0.06±0.02	0.14±0.05	0.16±0.04
-Cr+C	0.009±0.0007	2.11±0.16	0.58±0.02	0.20±0.010	0.28±0.01	0.65±0.04	0.65±0.03
+Cr-C	0.003±0.0008	0.45±0.17	0.11±0.02	0.05±0.010	0.07±0.02	0.12±0.05	0.11±0.04
+Cr+C	0.008±0.0008	1.90±0.17	0.56±0.02	0.17±0.010	0.28±0.02	0.68±0.05	0.52±0.04
Source of Variation			P Values				
Cr	0.96	0.44	0.39	0.25	0.98	0.95	<0.03
C	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cr x C	0.47	0.66	0.80	0.29	0.71	0.62	0.25

[1]Values are least squares means ± SEM (Cr, n = 18-20; C, n = 19).

TABLE 5

^{14}C-Ascorbate in tissues, urine, and expired as carbon dioxide by guinea pigs 6 h after oral dose[1]

	Plasma	Adrenal	Liver	Brain	Spleen	Testes	Kidney	Urine	Carbon Dioxide
				Percentage of ^{14}C dosed					
-Cr	1.245±0.069	0.074±0.010	2.14±0.20	0.057±0.004	0.16±0.01	0.049±0.006	0.45±0.04	3.00±0.52	7.14±0.50
+Cr	1.321±0.069	0.074±0.009	2.05±0.20	0.039±0.004	0.10±0.01	0.042±0.006	0.40±0.04	3.15±0.52	5.67±0.49
-C	1.227±0.071	0.062±0.009	1.78±0.20	0.046±0.004	0.16±0.01	0.050±0.006	0.42±0.04	2.96±0.53	6.68±0.48
+C	1.339±0.067	0.086±0.009	2.42±0.20	0.051±0.003	0.09±0.01	0.041±0.006	0.44±0.04	3.18±0.50	6.13±0.50
-Cr-C	1.037±0.103	0.066±0.014	1.90±0.30	0.055±0.005	0.22±0.02	0.065±0.009	0.45±0.05	2.13±0.78	7.03±0.71
-Cr+C	1.454±0.092	0.083±0.013	2.38±0.27	0.060±0.004	0.11±0.02	0.034±0.009	0.46±0.05	3.86±0.69	7.25±0.71
+Cr-C	1.417±0.097	0.057±0.012	1.66±0.27	0.037±0.005	0.11±0.02	0.036±0.009	0.40±0.05	3.79±0.73	6.32±0.67
+Cr+C	1.224±0.097	0.090±0.012	2.46±0.28	0.042±0.005	0.09±0.02	0.048±0.009	0.41±0.05	2.50±0.73	5.02±0.71
Source of Variation				P Values					
Cr	0.45	0.95	0.76	<0.001	<0.0010	0.44	0.33	0.84	<0.05
C	0.26	0.06	<0.03	0.25	<0.005	0.33	0.82	0.76	0.45
Cr x C	<0.004	0.54	0.59	0.92	<0.01	<0.03	0.99	<0.05	0.29

[1]Values are least squares means ± SEM (Cr, n = 18-20; C, n = 19).

TABLE 6

Tissue manganese concentration (ng/g dry wt) of guinea pigs fed diets with or without added Cr and given marginal ascorbate (1 mg/day) or supplemented with ascorbate (10 mg/day)[1]

	Bone	Liver	Brain	Adrenal	Testes	Kidney	Spleen
-Cr	273±28	6323±585	1245±55	4675±500	3846±327	5392±280	765±47
+Cr	327±28	8048±581	1147±55	3941±500	4157±325	4355±278	895±47
-C	292±28	7804±599	1107±57	3585±512	3381±335	4909±287	899±48
+C	308±27	6568±567	1285±54	5031±485	4621±317	4838±271	761±46
-Cr-C	224±42	6583±872	1245±83	4036±727	3514±487	5648±417	839±70
-Cr+C	322±38	6064±780	1245±74	5314±687	4178±436	5136±373	691±62
+Cr-C	361±39	9026±822	968±78	3134±729	3249±459	4170±393	959±66
+Cr+C	293±39	7072±822	1326±78	4748±687	5065±459	4541±393	831±66
Source of Variation			P Values				
Cr	0.18	<0.05	0.22	0.30	0.50	<0.02	0.06
C	0.70	0.14	<0.03	<0.05	<0.01	0.86	<0.05
Cr x C	<0.05	0.39	<0.03	0.81	0.22	0.27	0.87

[1]Values are least squares means ± SEM (Cr, n = 18-20; C, n = 19).

TABLE 7

Tissue iron concentration (ug/g dry wt) of guinea pigs fed diets with or without added Cr and given marginal ascorbate (1 mg/day) or supplemented with ascorbate (10 mg/day)[1]

	Bone	Liver	Brain	Adrenal	Testes	Kidney	Spleen
-Cr	36±3	208±18	83±4	221±16	157±14	128± 7	1136± 87
+Cr	30±3	193±18	84±4	212±16	142±14	132± 7	1137± 86
-C	36±3	159±19	75±4	210±16	122±15	114± 7	1008± 89
+C	30±3	242±18	92±4	224±15	178±14	147± 7	1266± 84
-Cr-C	38±4	158±27	77±5	215±23	124±21	113±10	1001±129
-Cr+C	33±4	258±25	80±5	227±21	190±19	143± 9	1272±115
+Cr-C	34±4	160±26	73±5	205±22	120±20	114±10	1014±122
+Cr+C	26±4	226±26	94±5	220±22	165±20	150±10	1260±122
Source of Variation			P Values				
Cr	0.17	0.57	0.92	0.71	0.47	0.68	0.99
C	0.12	<0.01	<0.01	0.54	<0.01	<0.01	<0.04
Cr x C	0.67	0.52	0.42	0.95	0.60	0.77	0.92

[1]Values are least squares means ± SEM (Cr, n = 18-20; C, n = 19).

TABLE 8

Tissue chromium concentration (ng/g dry wt) of guinea pigs fed diets with or without added Cr and given marginal ascorbate (1 mg/day) or supplemented with ascorbate (10 mg/day)[1]

	Bone	Liver	Brain	Adrenal	Testes	Kidney	Spleen
-Cr	378±108	116±77	95±41	229±89	755±135	53±157	56±28
+Cr	416±104	451±74	230±35	518±86	309±143	741±156	175±27
-C	566±108	313±79	173±41	329±89	469±138	483±161	125±28
+C	228±104	254±72	152±35	418±85	594±140	310±152	106±26
-Cr-C	511±157	123±118	117±66	177±129	682±201	51±234	75±43
-Cr+C	245±147	110±99	73±49	280±121	827±160	53±210	38±36
+Cr-C	621±147	502±104	229±49	481±121	257±190	914±221	176±38
+Cr+C	211±147	399±104	231±49	555±121	360±215	567±221	176±38
Source of Variation			P Values				
Cr	0.80	<0.01	<0.02	<0.03	<0.04	<0.01	<0.01
C	<0.04	0.59	0.70	0.48	0.53	0.44	0.64
Cr x C	0.64	0.67	0.68	0.91	0.92	0.44	0.63

[1]Values are least squares means ± SEM (Cr, n = 18-20; C, n = 19).

CHAPTER VII

CHROMIUM AND ASCORBATE EFFECTS ON 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE IN GUINEA PIGS

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ABSTRACT

Supplemental chromium has sometimes decreased blood cholesterol; ascorbate deficiency has contributed to elevated serum cholesterol. Therefore, chromium and ascorbate depletion effects on 3-hydroxy-3-methylglutaryl coenzyme A were investigated in guinea pigs. Weanling male guinea pigs were randomly assigned to groups in a 2x2 factorial design. Animals were fed 1 mg ascorbate per day (-C) or 10 mg ascorbate per day (+C). Diets contained <.07 ug/g chromium or were supplemented with 2 ug/g chromium as $CrCl_3$ and were fed for 21 weeks. Activity of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG CoA) was determined using 3-^{14}C-HMG-CoA with ^{3}H-mevalonate as an internal standard. Tissue minerals were determined by flame

or graphite furnace atomic absorption spectroscopy. Body weight was not significantly affected by dietary treatments. Plasma cortisol and cholesterol were not affected by chromium or ascorbate treatments. Iron in the liver was increased by ascorbate supplementation. Cr supplementation decreased hepatic iron. An interaction between chromium and ascorbate affected activity of HMG CoA reductase, the rate limiting enzyme in cholesterol biosynthesis. In ascorbate depleted animals, chromium supplementation increased HMG CoA reductase activity, while in ascorbate supplemented animals, Cr supplementation decreased activity. The mean dpm values per mg protein from ^{14}C mevalonate for -Cr-C, -Cr+C, +Cr-C, and +Cr+C groups were 8998, 14,947, 14,500, and 7628, respectively.

Key Words: Chromium, ascorbic acid, 3-hydroxy-3-methylglutaryl coenzyme A reductase, iron

INTRODUCTION

Chromium affects lipid metabolism under some experimental conditions but mechanisms for its effects have not been resolved. Serum cholesterol was elevated in rats fed sucrose as a carbohydrate source; rats supplemented with chromium had lower serum cholesterol (1). Feeding 1 ug chromium/g diet decreased serum cholesterol in male rats, while 5 ug/g chromium lowered serum cholesterol in female rats (2). Potassium chromate injected intraperitoneally

into rabbits fed a high cholesterol diet reduced the size of aortic plaques and decreased aortic cholesterol (3).

Curran (4) demonstrated in vitro that trivalent chromium enhanced the synthesis of cholesterol and fatty acids in rat liver. When chicks were fed 20 ug Cr/g diet the chromium increased ^{14}C incorporation into hepatic fatty acids compared to controls (5). Xu and co-workers reported increased plasma cholesterol in guinea pigs supplemented with 4 ug trivalent chromium/g diet versus 2 ug Cr/g (6), yet chromium has not been implicated in the function of 3-hydroxy-3-methylglutaryl coenzyme reductase, the rate limiting step in cholesterol biosynthesis.

Ascorbate deficiency also has contributed to elevated serum cholesterol. In chronic vitamin C deficiency, guinea pigs produced less bile acid and had higher hepatic and serum cholesterol than controls (7) while serum cholesterol elevation was exacerbated by combined chromium and vitamin C deficiencies in guinea pigs (8).

Interaction of chromium and ascorbate in cholesterol synthesis has not been investigated. The purpose of this study was to determine if hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, the rate limiting step in cholesterol synthesis (9,10), would be affected by chromium depletion and/or chronic vitamin C deficiency.

MATERIALS AND METHODS

Weanling male Hartley guinea pigs weighing approximately 200 g were randomly assigned to chromium and/or ascorbate treatment groups in a 2x2 factorial design. Animals were fed 1 mg ascorbate/d (-C) or 10 mg ascorbate/d (+C). Diets contained <.070 ug Cr/g diet (-Cr) or were supplemented with 2 ug Cr from $CrCl_3$/g diet were fed for 21 weeks. Diets and mineral mix were formulated in our laboratory after testing individual components for chromium contamination (Table 1).

Animals were housed in plexiglass cages with floors of plastic grating. Food and distilled deionized water were available ad libitum in ceramic cups. Guinea pigs were weighed weekly. Two weeks before necropsy, animals in the minus ascorbate group were decreased to 0.5 mg ascorbate/day. For last 11 days of the experiment, animals were fed at 0200 h and food was removed at 1400 h so that 3-hydroxy-3-methylglutaryl coenzyme A reductase activity would peak between 0800-1100 h. At 0200 h prior to autopsy, guinea pigs were fed 15 g of diet. Six to seven hours later, animals were anesthetized with halothane gas and exsanguinated by cardiac puncture using heparinized syringes. Plasma was frozen for cholesterol and cortisol analysis.

The guinea pig livers were rapidly excised, and approximately 1 g was weighed, wrapped in foil, and placed on ice until homogenization in 5 volumes of Krebs-Ringer

phosphate buffer (pH 7.4) in a mechanical homogenizer. The homogenate was centrifuged at 15000 x g for 20 min. and the supernatant was recentrifuged at 15000 x g for 20 min. The resultant supernatant was centrifuged at 105,000 x g for 2 h. Supernatant protein was measured by Brilliant Blue G-250 (US Biochemical Corporation, Cleveland OH). Activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase was measured in a reaction mixture (1 mL) containing glucose 6-phosphate (10 mM), NADP (1 mM), EDTA (1 mM), dithiothreitol (1 mM), potassium phosphate buffer pH 7.4 (0.1 M), glucose 6-phosphate dehydrogenase (2 units), 100 ug protein, and [3-14C] HMG-CoA (7.9×10^4 dpm). After a 1 h incubation at 37° C, HCl (200 ul) was added to stop the reaction, and the samples were frozen.

Aliquots (100 ul) were chromatographed on a 0.5 x 5 cm Dowex-1-formate column (Bio-Rad Laboratories, Richmond, Ca) with ^{3}H-Mevalonate (2×10^5 dpm) as an internal standard. Mevalonolactone was eluted with 5 ml water. A 100 ul aliquot of the eluant containing mevalonolactone was counted in Insta-Gel (5ml) using the ^{3}H and ^{14}C channels of a Tri-Carb 1900CA Liquid Scintillation Analyzer (Packard, Laguna Hills, CA) Final values were expressed as DPM/mg protein and were corrected based on ^{3}H-mevalonate recovery.

Tissues samples frozen in liquid nitrogen at necropsy were homogenized in 5 ml 10% cold trichloroacetic acid, deproteinized and analyzed for vitamin C within 4 hr using the ferrozine method.

Cholesterol was measured in serum from blood drawn from the toenail at week 14 and from plasma at necropsy. Cholesterol concentrations were measured enzymatically (Method #352, Sigma Chemical Co., St. Louis, Mo) (11). Cortisol in the plasma was analyzed by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) and cortisol concentrations were computer generated from a logit-log calibration curve.

For dietary chromium analysis, samples of diets (200-250 mg) were ashed in acid-washed borosilicate glass tubes in a muffle furnace with no exposed metal heating elements (Lindberg, Watertown, WI) using the method of Hill and co-workers (12). Samples were diluted with 0.5% nitric acid (double-distilled; G. Fredrick Smith, Columbus, OH) and analyzed for manganese and chromium using a Perkin-Elmer Model 5100 PC atomic absorption spectrophotometer with a graphite furnace and Zeeman background correction (Perkin Elmer, Norwalk, CT).

Tissue samples were cut with glass knives in a clean air hood. Drying and ashing procedures for tissues were identical to those for diet. Chromium and manganese were determined by graphite furnace atomic absorption spectroscopy (AAS); iron was determined by flame AAS.

Data were analyzed as a 2x2 factorial design using the generalized linear model procedure of the Statistical Analysis System (SAS).

RESULTS

Body weight was not affected by treatment (Table 2); nor were there differences in tissue weights except heart. Guinea pigs fed the chromium depleted diet had lower heart weights. Liver weight tended to be higher in ascorbate depleted guinea pigs ($p<0.08$).

Tissue ascorbate was increased by ascorbate supplementation ($p<0.0001$). Ascorbate concentrations of adrenal, kidney, liver, and testes for animals depleted to 0.5 mg ascorbate per day were 0.16, 0.01, 0.02 and 0.07, respectively and values for animals fed 10 mg/d were 1.53, 0.14, 0.27 and 0.46, respectively. Chromium did not affect tissue ascorbate in this study.

Serum cholesterol at week 14 was not affected by chromium or ascorbate treatment (Table 3). Plasma cholesterol at necropsy (week 21) also was not affected by chromium treatment, but cholesterol values tended to be elevated with ascorbate depletion ($p<0.10$). Likewise, plasma cortisol values were not affected by chromium or ascorbate treatments.

Chromium in the liver and kidney were increased by chromium supplementation ($p<0.03$) but were not affected significantly by ascorbate treatment (Table 4). There was high variability in chromium of the liver and neither chromium or ascorbate treatments affected hepatic chromium. Liver and kidney manganese values were not affected by chromium or ascorbate treatment. Iron in the kidney was

increased by ascorbate ($p<0.0001$) and was decreased by chromium supplementation ($P<0.04$).

There was an interactive effect ($p<0.0002$) of ascorbate and chromium on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity (Figure 1). In ascorbate depleted animals, chromium supplementation increased HMG CoA reductase activity, while in ascorbate supplemented animals, Cr supplementation decreased activity.

DISCUSSION

Guinea pigs in this experiment did not demonstrate growth differences due to chromium or ascorbate treatments. Tissue weights were not affected except for heart which was decreased by the chromium depletion diet. The guinea pigs had an initial weight of 205 g; growth effects due to chromium depletion were noted previously in guinea pigs with mean initial weights of 165 g. Chromium content of diet used in this study was similar (<0.070 ug/g) to the one used in the previous experiment (<0.060 ug/g); the guinea pigs in the present experiment may have higher initial chromium stores due to longer time with the dams or they may have received laboratory chow diets for a few days before shipping. However, in comparison to animals from the previous experiment, these animals had lower hepatic and kidney chromium.

The 0.5 mg/d ascorbate given to these animals the last 2 wk before autopsy resulted in lower tissue ascorbate than in

animals receiving 1 mg ascorbate in our previous study (chapter VI). For example, values in mg/g for animals receiving 0.5 mg/d ascorbate in the adrenal, kidney, liver and testes were 0.16, 0.02, 0.02, and 0.07, respectively, while values for animals receiving 1 mg ascorbate were 0.48, 0.05, 0.07, and 0.13 respectively.

Chromium supplementation increased chromium in both kidney and liver. Increased chromium in kidney has been reported with chromium supplementation (13) and higher chromium levels of both liver and kidney were found in the previous experiment. Manganese concentrations in liver and kidney were not affected by either chromium and ascorbate. Hepatic iron was significantly increased by ascorbate and decreased by chromium supplementation. An increase in iron due to ascorbate supplementation has been reported in male (25 mg ascorbate/100 g bw) (14) and female guinea pigs (225 mg ascorbate/d) (15). A decrease in iron due to chromium supplementation has not been reported previously. However, iron deficient animals absorbed more chromium than iron-supplemented controls and iron depressed chromium binding to transferrin in vitro (16). In inverted sac experiments iron appreciably depressed ^{51}Cr transport (17).

When serum cholesterol is lowered by drugs, cholesterol synthetic enzymes increase their activity. In this study HMG CoA reductase activity in the presence of either supplemental vitamin C or chromium was elevated. However,

when both ascorbate and chromium (+Cr+C) were present, values comparable to those in the -Cr-C group resulted.

Neither plasma cholesterol nor cortisol were affected by chromium or ascorbate depletion. As 3-hydroxy-3-methylglutaryl coenzyme A reductase activity is affected by circulating cholesterol (18), the variation in HMG CoA reductase activity cannot be explained by the cholesterol data. Because cortisol values are not different, it does not appear cortisol affected HMG CoA activity in this study. However, cortisol may be necessary for maximal functioning of HMG CoA reductase (19). Increased cortisol values were found in chromium depleted guinea pigs in experiment 6, and chronic administration of cortisol induced hyperinsulinemia in mice (20). A consequence of hyperinsulinemia is a decrease in number of insulin-receptor sites (21).

Insulin appears to have a dominant role in the regulation of the phosphorylation state of HMG CoA reductase (22). Insulin signaled net dephosphorylation enhancing reductase activity and glucagon signaled phosphorylation depressing reductase activity (23). Neither glucagon nor insulin was measured in these guinea pigs, but Banergee and Ghosh reported that ascorbate deficiency resulted in hypoinsulinism (24). Chromium in the form of $CrCl_3$ inhibited insulin secretion by isolated pancreatic islets (25). In cultured lymphocytes, insulin binding was inversely proportional to insulin concentration (21). Mertz reported that chromium potentiates the action of insulin

(26). HMG CoA reductase activity as a result of the experimental treatments may reflect effects of circulating insulin and chromium.

HMG CoA reductase activity is depressed in guinea pigs by both excessive and deficient ascorbic acid (29). Ascorbic acid has a hypocholesterolemic effect only within a certain range of intakes (30). Ascorbate may be protected from oxidative destruction in chromium-supplemented animals. In experiment 6 there was less $^{14}CO_2$ from ^{14}C-ascorbate oxidation expired by chromium supplemented animals.

Brown, Dana, and Goldstein (31) reported that apolipoprotein B, a component of low density lipoproteins and very low density lipoproteins was involved in the suppression of HMG CoA reductase. Chronic vitamin C deficiency lowered catabolic rate of low-density lipoproteins in guinea pigs and deceased LDL receptors on the surface of liver cells (32).

Ascorbic acid is required for the hydroxylation of cholesterol to form bile acids (33). The 7 alpha-hydroxylase activity was markedly reduced in ascorbate-deficient animals. Both biliary obstruction (34) and biliary diversion (18) increased hepatic bile acids and resulted in increased cholesterol synthesis. However, when bile acids were fed at 1% of the diet, HMG CoA reductase activity was reduced (35). Thus hepatic bile acids or their enterohepatic circulation may regulate HMG CoA reductase.

Catacholamines (36), cortisol (19), thyroid (37) and insulin (38) increased, whereas glucagon (19) and hydrocortisone (19) depressed cholesterol synthesis. Measurement of fecal bile acids, plasma insulin, glucagon, and thyroid would provide important clues to factors controlling HMG CoA reductase activity. These will be analyzed in our laboratory in future studies.

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TABLE 1

Composition of the Diet[1]

Component	g/kg
Casein	300
Arginine	3
Dextrose	379
Celufil[2]	150
Corn oil	70
Vitamin mix[3] (without ascorbic acid)	22
Potassium acetate	35
Mineral mix[4] (without chromium)	50

[1]By analysis the -Cr diet contained <70 ppb Cr and the +Cr diet contained 2 ppm.
[2]US Biochemical Corp.
[3]The vitamin mix contained per kg (in g except as noted) alpha-tocohperol 5.0; choline chloride 75.0; d-calcium pantothenate, 3.0; inositol, 5.0; menadione, 2.25; niacin, 4.5; paraaminobenzoic acid, 5.0; pyridoxine HCl, 1.0; riboflavin, 1.0; thiamine HCl, 1.0; vitamin A acetate, 900,000 units; calciferol (D_2) 100,000 units; biotin, 20 mg; folic acid, 90 mg; and vitamin B_{12}, 1.35 mg.
[4]The mineral mix contained (g/kg): $CaHPO_4$, 600.0; NaCl, 80.0; MgO, 100.0; $ZnCO_3$, 0.9; $MnCO_3$, 1.8; $CuCO_3 \cdot Cu(OH)_2$ H_2O, 0.25; KIO_3, 0.035; $NaSeO_3 \cdot 5H_2O$, 0.0044; and $FeSO_4$, 1.4235.

TABLE 2

Body and tissue weights of guinea pigs fed diets with or without added Cr and given minimal ascorbate (.5 mg/day) or supplemented with ascorbate (10 mg/day)

Treatment	Body Weight	Heart	Liver	Kidney	Testes
	g	g	g	g	g
-Cr	687±23	1.74±0.07	23.40±1.39	5.11±0.17	1.73±0.14
+Cr	719±26	2.02±0.08	23.87±1.61	5.29±0.20	1.97±0.16
-C	694±22	1.97±0.06	25.53±1.31	5.23±0.16	1.78±0.13
+C	713±28	1.80±0.08	21.74±1.67	5.18±0.21	1.92±0.17
-Cr-C	700±31	1.95±0.09	26.87±1.85	5.34±0.23	1.80±0.19
-Cr+C	674±35	1.53±0.10	19.93±2.08	4.89±0.26	1.67±0.21
+Cr-C	686±31	1.98±0.09	24.20±1.86	5.12±0.23	1.77±0.19
+Cr+C	751±44	2.06±0.13	23.55±2.63	5.47±0.33	2.17±0.27
Analysis of Variance			p values		
Cr	0.38	<0.01	0.82	0.49	0.29
C	0.58	0.10	0.08	0.85	0.54
Cr x C	0.21	<0.03	0.15	0.14	0.22

TABLE 3

Blood cholesterol and cortisol of guinea pigs fed diets with or without added Cr and given minimal ascorbate (.5 mg/day) or supplemented with ascorbate (10 mg/day)

Treatment	Cholesterol in serum (week 14)	Cholesterol in plasma (week 21)	Cortisol in plasma (week 21)
	mg/dl	mg/dl	ng/dl
-Cr	77±4	67±3	352±28
+Cr	68±4	62±4	350±32
-C	75±4	68±3	372±27
+C	69±5	60±4	330±33
-Cr-C	81±5	73±5	366±39
-Cr+C	72±6	61±5	337±41
+Cr-C	69±5	64±4	377±37
+Cr+C	67±7	59±6	322±52
Analysis of Variance		p values	
Cr	0.15	0.28	0.95
C	0.32	0.10	0.33
Cr x C	0.51	0.53	0.76

TABLE 4

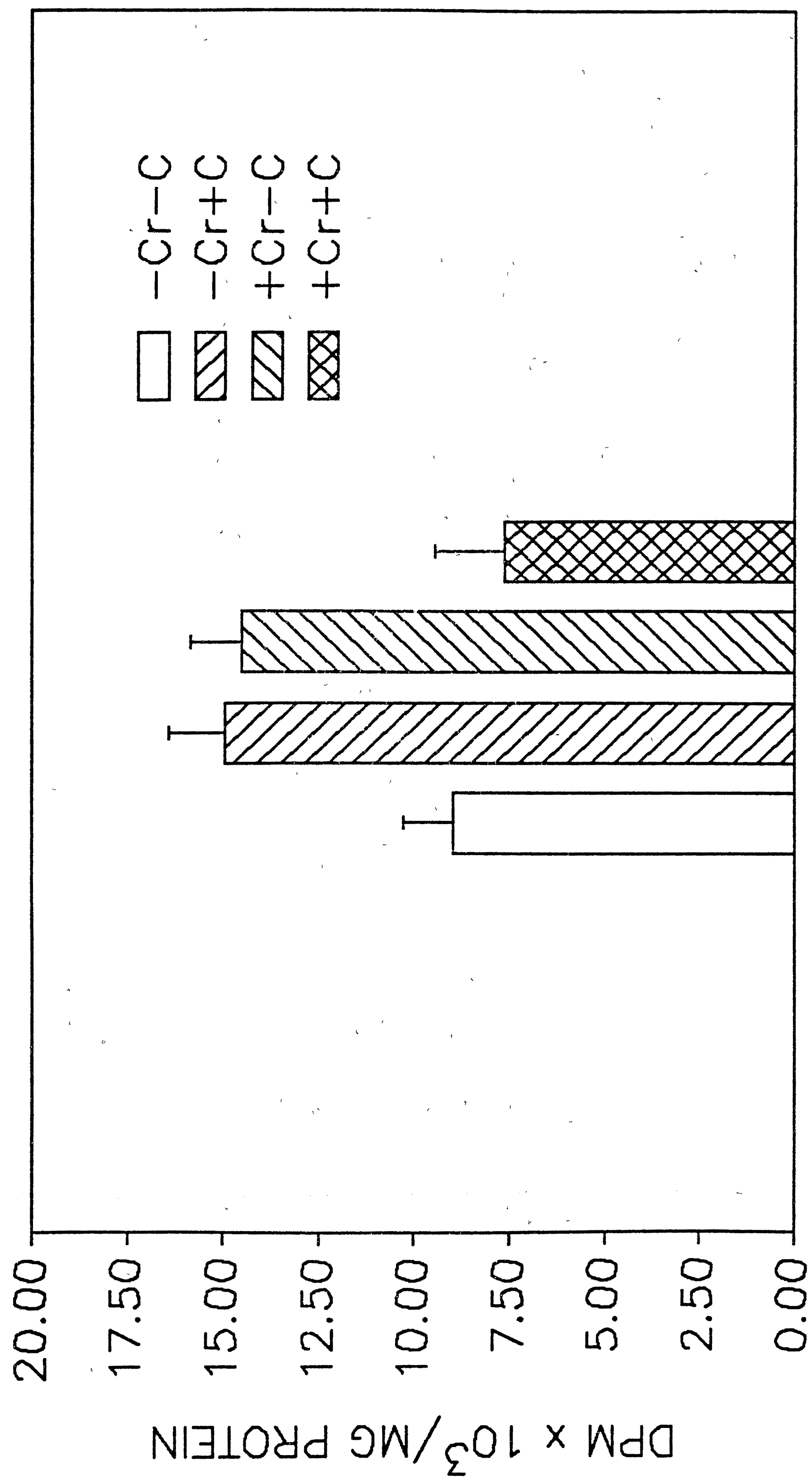
Liver and kidney minerals of guinea pigs fed diets with or without added Cr and given minimal ascorbate (.5 mg/day) or supplemented with ascorbate (10 mg/day)

Treatment	Liver[1]	Kidney	Liver	Kidney	Liver
	Chromium ng/g		Manganese ug/g		Iron ug/g
-Cr	20±16	33± 9	5.13±.34	5.49±.51	163± 8
+Cr	45±13	67±10	4.38±.39	4.44±.59	138± 9
-C	40±10	50± 8	4.64±.32	4.64±.48	124± 7
+C	26±13	50±10	4.89±.40	5.29±.62	178± 9
-Cr-C	18±15	29±11	4.83±.45	4.74±.68	130±10
-Cr+C	22±16	37±13	5.43±.50	6.25±.77	195±11
+Cr-C	62±15	71±11	4.44±.45	4.53±.68	117±10
+Cr+C	29±21	63±16	4.33±.63	4.34±.97	160±14
Analysis of Variance			p values		
Cr	0.03	<0.02	0.15	0.19	<0.04
C	0.42	0.99	0.64	0.41	0.0001
Cr x C	0.06	0.55	0.50	0.29	0.36

[1] Data for liver chromium was not normally distributed and log transformations were formed.

Legend for Figure

FIG 1. Activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase of guinea pigs fed diets with or without added Cr and given marginal ascorbate (.5 mg/day) or supplemented with ascorbate.



DPM x 10³/MG PROTEIN
20.00
17.50
15.00
12.50
10.00
7.50
5.00
2.50
0.00
−Cr−C
−Cr+C
+Cr−C
+Cr+C
HMG CoA REDUCTASE ACTIVITY

CHAPTER VIII

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

This research evaluated nutrient and drug interactions affecting chromium retention and excretion and the effects of chromium and vitamin C depletion and supplementation on cholesterol synthesis, on tissue trace minerals, on vitamin C retention, and on some indices of growth including hydroxyproline, creatinine, and blood urea nitrogen.

Five objectives and hypotheses are listed in the introduction of this dissertation. Each will be addressed individually; then other general conclusions and recommendations will be discussed.

Hypothesis one stated that there will be no statistically significant effects of carbohydrate (glucose, fructose, sucrose or starch), chromium supplementation, or genetic obesity on tissue chromium or on retention and urinary excretion of ^{51}Cr from $^{51}CrCl_3$.

A load of 2 mg starch/g body weight increased ^{51}Cr retention compared with loads of glucose, fructose or sucrose in all tissues analyzed except kidney. Chromium concentrations generally were higher in tissues from animals consuming the starch diet than in tissues from

those consuming diets containing simple carbohydrates; differences paralleled data 2 h after the $^{51}CrCl_3$ dose. Chromium concentrations after 26 d of feeding the experimental diets were higher in the testes, spleen and bone and lower in the liver of obese mice compared to values in lean mice. Chromium supplementation increased chromium in the kidney ($p<0.0005$) and bone ($p<0.0001$) in this study. Based on these results, the first null hypothesis is rejected.

Hypothesis two stated that there will be no statistically significantly differences in ^{51}Cr tissue accumulation or excretion in rats dosed with $^{51}CrCl_3$ and vitamin C or calcium carbonate antacid compared with rats dosed with water.

Twenty-four hours after dosing, ^{51}Cr in cumulative urine was lower in the group dosed with the calcium carbonate antacid than in the groups dosed with vitamin C or water. Accumulation of ^{51}chromium in the kidney, testes, and spleen was lower in rats dosed with the antacid than in those dosed with vitamin C or water. In blood at 3 hr, ^{51}chromium was higher in rats dosed with either vitamin C or water than with the calcium carbonate antacid. Hypothesis two was rejected based on these results.

Hypothesis three stated there will be no statistically significant differences in ^{51}Cr in tissues, blood, and urine of guinea pigs deprived of chromium and/or vitamin C compared with those fed adequate chromium and vitamin C.

With vitamin C depleted animals, following a glucose load, urinary excretion of ^{51}chromium was higher than in glucose loaded vitamin C adequate animals. Tissue ^{51}chromium at 3 h after the dose was not affected by vitamin C status. Animals fed -Cr diets had higher ^{51}Cr in blood and liver than those fed +Cr diets but urinary excretion of ^{51}Cr was not affected by prior chromium intake. Based upon these results null hypothesis three was rejected.

Hypothesis four stated there will be no statistically significant differences in ^{14}C in blood, tissues, urine and expired carbon dioxide from a ^{14}C vitamin C dose and no differences in tissue mineral concentrations, tissue vitamin C, blood urea nitrogen, cortisol, and urinary excretion of hydroxyproline and creatinine due to chromium/vitamin C status.

In experiment 4, beginning at week 13, animals fed the Cr depletion diet weighed less than animals fed diet supplemented with 2 ug Cr/g. There were no differences in urinary hydroxyproline or creatinine values at week 8 or week 20 due to chromium treatment. Vitamin C status, likewise, did not affect creatinine or hydroxyproline excretion. Cortisol in urine at week 20 was not affected by Cr or vitamin C treatment. The plasma cortisol of animals restrained in metabolic cages for six hours was higher ($p<0.05$) in -Cr animals, but was not affected by vitamin C treatment. In animals supplemented with 10 mg/d ascorbate, there was an effect of chromium on plasma urea nitrogen.

The tissue and plasma vitamin C of animals in experiment 4 was increased by vitamin C supplementation but was not affected by Cr treatment except for testes in which vitamin C decreased with Cr supplementation. Expired $^{14}CO_2$ from a ^{14}C-ascorbate dose was greater in -Cr animals and was not affected by vitamin C treatment. There was increased ^{14}C in the brain of guinea pigs fed a low Cr diet. Animals supplemented with 10 mg/d of vitamin C retained more ^{14}C in the liver than animals which received 1 mg/d vitamin C. There were significant interactive effects of Cr and vitamin C on ^{14}C in testes, spleen, urine, and plasma.

Tissue minerals of animals in experiment 4 were affected by treatment. Copper in the kidney was decreased by the 10 mg/d vitamin C supplementation. Vitamin C supplementation resulted in increased iron concentrations in the liver, brain, testes, kidney, and spleen. No differences were seen in tissue copper, zinc, or iron concentrations due to chromium treatment. Manganese was higher ($p<0.05$) in liver and lower ($p<0.02$) in kidney of chromium supplemented animals. Splenic manganese tended to increase ($p<0.06$) with Cr supplementation. Vitamin C supplementation increased manganese in the adrenal ($p<0.05$) and testes ($p<0.01$) and decreased manganese in the spleen ($p<0.05$). Interactive effects of chromium and vitamin C treatments were found on manganese concentrations in bone and brain. Based on these results, null hypothesis 4 was rejected.

Hypothesis 5 stated that there will be no statistically significant differences in hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity due to chromium depletion and/or chronic vitamin C deficiency. Although no differences in plasma cholesterol were found, a significant interaction of Cr and vitamin C on HMG CoA reductase activity was found. Therefore, null hypothesis 5 also was rejected.

Conclusions

The conclusion from experiment 1 was that a carbohydrate load of starch facilitated ^{51}Cr uptake compared with a carbohydrate load of sucrose, fructose or glucose. Similarly, starch-fed mice had higher tissue chromium than did fructose-, glucose-, or sucrose-fed mice. Thus, the type of dietary carbohydrate affected chromium absorption and retention. Genetic obesity and chromium supplementation also affected tissue chromium concentration.

In experiment 2, at the dose level used, a calcium carbonate antacid reduced ^{51}chromium in blood at 3 hr and in spleen, kidney, testes, and urine of rats compared with animals intubated with vitamin C or water. Compared with water or calcium carbonate, vitamin C increased cumulative urinary ^{51}chromium excretion at 24 hr after dosing. This experiment illustrated that absorption of ingested chromium chloride is low and suggested that drugs may affect chromium uptake.

An effect of prior chromium status on ^{51}Cr retention in blood and liver (experiment 3) has not been seen in shorter studies; however, in this 24 week experiment, chromium deprivation enhanced the uptake of ^{51}Cr from a dose of $^{51}CrCl_3$. It may no longer be appropriate to maintain that past dietary chromium has no effect on retention and excretion of chromium. The finding of increased urinary excretion of ^{51}Cr in response to a glucose load in vitamin C depletion has not been reported previously.

The lower body weight of -Cr animals in experiment 4 supports the essentiality of Cr for animal growth. An effect of dietary Cr and ascorbate on protein metabolism is indicated by the interaction of Cr and vitamin C on plasma urea nitrogen. Elevated cortisol was found in plasma of chromium depleted animals confined to cages for 6 h, but no differences were found in urinary cortisol collected prior to confinement.

The alterations in tissue vitamin C and distribution of ^{14}C-ascorbate as affected by Cr and vitamin C in immobilization stress were measured in experiment 4. Plasma cortisol after guinea pigs had been immobilized for 6 hr, was double reported normal values for these animals. Tissue vitamin C was not affected by Cr treatment except for testes which had higher vitamin C concentration in animals fed low Cr diets. The expired $^{14}CO_2$ of all groups was at the lower end of the reported range. The fact that -Cr animals expired more than +Cr animals, suggests that Cr

supplementation may protect vitamin C from utilization or oxidative destruction. Chromium and vitamin C status of stressed animals affected ^{14}C tissue distribution and excretion.

In experiment 4, liver manganese was higher ($p<0.05$) and kidney manganese was lower ($p<0.02$) with Cr supplementation. Tissue manganese also reflected ascorbate supplementation. Higher Mn in adrenal ($p<0.05$) and testes ($p<0.01$) and lower Mn in spleen ($p<0.05$) were observed in animals receiving 10 mg ascorbate. Vitamin C increased iron concentrations of several tissues and decreased kidney copper and bone chromium. Cr supplementation increased chromium values in several tissues but did not affect either iron or copper in the tissues analyzed. Thus, specific minerals in tissues may be impacted by dietary chromium and/or vitamin C.

Increased activity of HMG CoA was observed in experiment 5 in the presence of either vitamin C or in the presence of chromium, but plasma cholesterol was not affected. However, when both chromium and vitamin C were present, they acted in a synergistic manner on HMG CoA reductase activity resulting in lower activity similar to the situation found when both Cr and vitamin C were absent. Vitamin C hydroxylates cholesterol in bile acid synthesis thus lowering serum cholesterol. The blood cholesterol values which were unaffected by Cr or vitamin C when HMG CoA was increased, suggest Cr may also be involved in the catabolism or synthesis of bile acids.

Recommendations

In experiment 1, starch facilitated ^{51}chromium uptake compared with a carbohydrate load of sucrose, fructose or glucose. Similarly, animals fed starch as 50% of their diet had higher tissue chromium than did fructose-, glucose-, or sucrose-fed mice. Urinary chromium excretion increases in subjects consuming simple sugars. Effects on urinary Cr excretion of soft drinks and other beverages such as orange juice containing simple sugars could be studied. Also, how lactose in milk affects chromium retention should be investigated. Furthermore, chromium may travel with water in the intestine. Future experiments might test various fiber sources capable of binding water which might slow the intestinal movement of chromium thus facilitating absorption.

At the dosage used in experiment 2, calcium carbonate antacid decreased ^{51}chromium retention in blood, spleen, kidney, and testes, and decreased urinary ^{51}chromium excretion. The dose was chosen to study acute effects of calcium carbonate on ^{51}chromium absorption. A future investigation of calcium carbonate should involve lower graded doses of the antacid. Also, the antacid should be given over time to investigate the chronic effects of antacid on chromium retention. Antacids composed of compounds other than calcium carbonate also should be tested. In vitro studies would be appropriate to determine

whether compounds such as antacids result in ^{51}chromium olation and subsequent precipitation.

The increased urinary excretion of ^{51}chromium after a glucose load in vitamin C depleted animals in experiment 3 may be due to brush border disaccharidases which proliferated when insulin was lacking or due to hormonal changes as a result of vitamin C deficiency. An interesting follow-up to experiment 3 would be to analyze disaccharidases and enzymes associated with glucose absorption to determine if enzyme activity is affected by vitamin C or chromium deficiency. A critical problem continues to be the determination of insulin values which may affect ^{51}chromium retention. Insulin was reported to be affected by ascorbate deficiency. The effect of a chromium deficient diet on insulin of guinea pigs is not known.

The ^{14}C data from experiment 4 reflects the physiological distribution of ^{14}C-ascorbate under stress. In order to determine effects of Cr and/or vitamin C depletion on the distribution of ^{14}C without the effect of stress, it would be necessary to repeat this experiment in a situation in which animals were not immobilized. Relationships between stress, cortisol, and urinary chromium excretion should be investigated. The effect of ACTH on ^{51}chromium retention and excretion would be an interesting project. Caffeine seems to have an similar effect as cortisol in the elevation of blood glucose. Another

interesting study would be evaluate the effects of caffeine on retention and excretion of ^{51}Cr.

The interaction of Cr and vitamin C on HMG CoA reductase in experiment 5 may be due to effects on bile acid metabolism. Therefore, a recommendation for future study would be to analyze fecal bile acids and hepatic cholesterol 7 alpha-hydroxylase activity to determine if bile synthesis as a result of chromium and/or ascorbate supplementation was influencing 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. It would also be desirable to determine if Cr and vitamin C may interactively affect low density and very low density lipoproteins and phospholipids. Due to the acknowledged role of insulin on HMG CoA reductase regulation, it is important to measure guinea pig insulin in response to the dietary treatments.

Work with animal models is essential to further ascertain the effects of dietary chromium and vitamin C on cholesterol and tissue vitamin C as well as to ascertain effects of selected nutrients and drugs on chromium retention and excretion. For such work to make great strides, however, it is essential to determine a biochemical measure of chromium status.

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