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CHROMIUM IN HUMAN NUTRITION

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CONTENTS

INTRODUCTION

Despite the acceptance of chromium (Cr) as a nutritionally essential trace element for animals and humans, information is sketchy and inconclusive regarding its biological functions. Cr is needed for appropriate glucose utilization (27, 44, 55, 83), and low Cr levels have also been related to abnormal lipid metabolism (1, 82, 87, 90, 95). But adequate measures of human Cr status, of the chemistry of Cr as a dietary component, or of its bioavailability are not yet available.

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There are three fundamental reasons for this. First, the fact that Cr is found in foods, the body, and the environment in nanogram amounts poses a dilemma. While it is extremely difficult to measure these very small amounts accurately in body tissues and fluids, Cr is so ubiquitous in the environment that incidental and often unrecognized contamination of a biological sample may increase the amount of Cr by much more than that in the original sample (109). Because much Cr contamination went undetected before 1978 (32), erroneously high Cr values were reported in the literature. Such values continue to be reported even today by investigators who are unaware of the full range of rigorous precautions that must be followed or who have inadequate instrumentation (104, 107).

Second, investigators using laboratory animals to document the effects of Cr depletion and repletion are hard pressed to find feed components that are Cr free, a must if clear signs of Cr deficiency are to be induced. Third, there is no direct measure of metabolic depletion or repletion of Cr. As a result, it is not surprising that many of the experimental data are inconsistent or conflicting.

Despite these obstacles, compelling evidence has accumulated that Cr is essential for normal glucose tolerance in both animals and humans and that it may also be a factor in lipid metabolism. In this chapter, we briefly review the evidence that Cr is an essential nutrient. Then we discuss a number of supplementation trials that have been conducted in people thought to be at risk of Cr deficiency, and we discuss the problems that impede this research. We then review current research findings in chromium metabolism. Finally, we examine the evidence that chromium may be marginal or inadequate in the diets of some people.

EVIDENCE THAT CHROMIUM (Cr) IS ESSENTIAL

Early Studies

The mammalian need for dietary Cr was first postulated by Schwarz & Mertz in 1957 (91). Their rats were fed stock diets but had impaired glucose tolerance for unknown reasons. When Cr-containing fractions of yeast or yeast concentrates were added to the diets, the tolerance to glucose improved. Cr^{3+} was identified as the active component of an organically complexed molecule that was called glucose tolerance factor (92). To investigate the effects of Cr, rats were deprived of dietary Cr; their glucose tolerance rates were impaired despite adequate insulin (59). When an intravenous dose of Cr^{3+} was given in a subsequent experiment, glucose disposal was restored to normal (58). In other studies, marked improvement in glucose tolerance were reported when Cr supplements such as brewer's yeast were given to a variety of laboratory animals, including rats (60, 83, 117), mice (100), and squirrel

monkeys (16), in various stages of Cr deprivation and with varying severity of diabetes or glucose intolerance. The organically complexed Cr in brewer's yeast was considered to be the biologically active form because a Cr-containing extract of brewer's yeast relieved the glucose intolerance of rats maintained on Cr-poor torula yeast. Mertz et al (12, 23, 55) postulated that Cr^{3+}, as a glucose tolerance factor, acts as a cofactor to bind insulin to membrane receptors in insulin-sensitive tissues, possibly by initiating sulfhydryl linkages. But because Cr was not given as a pure compound, but rather as extracts of either brewer's yeast or pork kidney homogenates, it was impossible to be sure whether the ameliorating effects of the extracts were due specifically to an organic Cr compound or whether they were due to some other substance in the extract.

Studies conducted by Schroeder et al (84, 88, 97, 98) in the 1960s to measure Cr in human tissues and diets suggested that dietary Cr deficiency may occur in humans, particularly as they age. Cr was the only heavy metal that decreased with age in body tissues (90, 97). These data are dubious, however, because the Cr concentrations are several hundred to a thousand times greater than those now measured in body tissues (103, 106, 111).

The analysis of Schroeder et al of Cr in foods also indicated extensive Cr losses due to refining and processing (85), and they found that Cr levels in Americans were lower than in people of third-world countries (88, 97, 98). Schroeder advanced the thesis that continued marginal or deficient Cr intakes, attributed to food losses and dietary habits (89), can have adverse health effects, particularly in the later decades of life, and can predispose some persons to maturity-onset diabetes mellitus and atherosclerotic disease (86). These data need to be reevaluated in view of the analytical limitations of the day.

These investigations and the concomitant animal data stimulated the study of the relationship between dietary Cr deficiency and glucose tolerance and lipid abnormalities. In a number of trials during the last two decades, Cr supplements were given to groups of people exhibiting abnormal glucose tolerance: patients receiving total parenteral nutrition (TPN), malnourished children, elderly persons, and diabetics. These studies, described below, have provided inconclusive data on whether preexisting dietary Cr deficiency can result in the impairment of glucose tolerance in humans fed orally.

Documentation of Human Cr Deficiency

PATIENTS RECEIVING TOTAL PARENTERAL NUTRITION In 1977, in the first documentation that Cr is needed for normal glucose tolerance in humans, Jeejeehboy et al (44) described a patient who developed hyperglycemia, weight loss, ataxia, and peripheral neuropathy after receiving TPN without Cr for 3½ years. After 250 μg of Cr as $CrCl_3$ were added, without insulin, to

the daily infusate for two weeks, glucose tolerance and neurologic function returned to normal and the lost body weight was regained. Normal glucose tolerance was then maintained on an intravenous dose of 20 μg of Cr daily.

A second and third report of successful Cr therapy in TPN patients followed in 1979 (24) and 1986 (10). In the second case, severe hyperglycemia occurred five months after the beginning of TPN therapy without added Cr. The patient was given 150 μg of Cr per day intravenously and glucose homeostasis was achieved within three days. Subsequently, the addition of 20 μg of Cr to the daily TPN solution maintained normal carbohydrate metabolism. In this case the rapid development of symptoms of Cr deficiency and the patient's prior rapid weight loss suggest that the Cr deficit probably antedated the TPN therapy (24).

The third patient developed unexplained hyperglycemia, glycosuria, and weight loss after almost seven months of TPN containing 6 μg of Cr per day (10). There were no signs of mental changes or peripheral neuropathy. The glucose intolerance and weight loss were reversed after 200 μg of Cr^{3+} were added to the daily infusate. The patient's glucose tolerance then remained normal on a home TPN preparation containing 26 μg of Cr per day. The investigators postulated that the rapid development of Cr depletion, despite the 6 μg of Cr in the daily infusate, was possible because this patient suffered excessive jejunostomy losses during hospitalization.

Each of the investigators reported "lower than normal" Cr values, but in none of these cases can the Cr measurements be considered accurate. Not only are the "normal" values from 20 to 100 times higher than those now recognized as normal (Table 1), but even the values reported to be "lower than normal" are far higher than the values in Table 1. Also, it is uncertain that even reliable measurements of Cr in serum or plasma reflect tissue levels or body stores (3, 55, 67). Thus, although these cases document that Cr deficiency can be identified retrospectively in patients whose unexplained hyperglycemia is corrected by Cr supplementation, they provide no correlation between levels of Cr in body tissues or fluids and the signs of Cr deficiency.

Table 1 Mean Cr levels in serum (S) or plasma (P) of fasted subjects

Number of subjects	Cr (μg/L)	Reference
76	(S) 0.13 ± 0.02 SEM[a]	3
30	(S) 0.24 ± 0.12 SD[b]	50
23	(P) 0.28 ± 0.09 SD	67
15	(S) 0.11 ± 0.07 SD	105
17	(S) 0.16 ± 0.08 SD	110a

[a]SEM = Standard error of the mean.
[b]SD = Standard deviation.

These TPN studies, accidental experiments, made apparent the following: first, that Cr is an essential nutrient in the human; second, that Cr is important in some manner in normal carbohydrate metabolism; third, that inorganic Cr given intravenously can, in a matter of days, reverse the abnormality in glucose metabolism so that, if conversion to a biologically active form is necessary, this conversion can occur quite rapidly; fourth, that 20 μg of inorganic Cr^{3+} per day is a quite adequate intravenous dose for maintenance of normal carbohydrate physiology.

SUBJECTS FED ORALLY Documenting Cr deficiency and its sequellae in orally fed people has been very difficult. Severe Cr deficiency is most unlikely because the very small amounts of dietary Cr needed are found not only in a variety of foods, but also as a contaminant from cookware (66). To induce a marginal dietary Cr deficiency probably requires habitually unsound eating habits with consequent deficits in other dietary elements. In such a situation, the effects of a marginal Cr deficiency are likely to be masked. Even when glucose intolerance is investigated, a cause-effect relationship cannot be inferred with any certainty, since one or more concomitant nutrient deficiencies may exert more profound effects on metabolism than the Cr deficiency. And, in contrast to TPN studies in which the complete documentation of nutrient intake can be obtained, oral dietary intakes cannot be documented accurately enough. The most limiting impediment is the absence of a reliable laboratory marker to diagnose Cr deficiency. These constraints have greatly complicated the design of controlled studies and the interpretation of data relating human Cr deficiency to glucose intolerance and lipid abnormalities.

Role of Cr in Lipid Metabolism

Several investigators have suggested that low tissue Cr levels may be linked to hyperlipidemia and atherosclerotic disease (56). This concept was first proposed by Schroeder (86), who reported that aortas of American subjects who died from cardiovascular disease were particularly Cr deficient, while aortas of healthy accident victims contained significantly more Cr. A similar report followed, indicating that the serum of patients with coronary artery disease had less than half as much Cr as did the serum of patients without coronary artery disease (63). As has already been noted, the measurements in both of these studies are unreliable and their replication using currently accepted techniques is needed in order to validate them.

The lipid question was addressed recently by Donaldson et al (19), who fed rats a high-sucrose, high-cholesterol, low-Cr semipurified diet and then added physiological levels of Cr to the diet of some of the animals. Dietary and tissue Cr concentrations were measured and the distribution of cholesterol and triglycerides in the blood was carefully assessed. These investigators were

unable to attribute differences in either glucose tolerance or plasma cholesterol concentrations solely to dietary Cr concentrations. They suggest that the divergent results between their experiment and earlier animal studies (2, 95) are due to important differences in a number of experimental factors. These include dietary factors unaccounted for in the earlier studies that may have caused or ameliorated glucose intolerance. For example, they postulated that the use in their study of corn oil to prevent fatty acid deficiency may have lowered cholesterol levels, while the use of cholic acid in one of the earlier studies may have exacerbated the hypercholesterolemia. Other likely reasons offered for variable results are stress-related lipid responses, caloric restriction, or pharmacologic, rather then physiologic, doses of Cr. Donaldson et al (19) urge that such factors be better identified and that careful measurements of tissue Cr levels be made if relationships between Cr deficiency and glucose intolerance and lipid abnormalities are to be demonstrated definitively in an animal model.

Supplementation Trials

For want of a direct measure of Cr status, investigators have selected groups of people with impaired glucose tolerance and plasma lipids that might be explained by (*a*) a deficiency of Cr in the diet, (*b*) an increased need for dietary Cr, or (*c*) increased Cr excretion. If the subjects' glucose tolerance and/or lipid levels improved following supplementation with Cr, then it was assumed, retrospectively, that Cr deficiency had been responsible for the initial glucose or lipid abnormality. Studies were undertaken with malnourished children in geographic areas reported to be Cr poor and with selected elderly subjects. Groups of diabetics were also supplemented because Cr deficiency has been suggested as an etiological factor in some cases of diabetes and because excessive Cr losses in the urine, due to the increased diuresis common in uncontrolled diabetes, may contribute to the glucose abnormality.

SUPPLEMENTS A major problem in the design and evaluation of these supplementation studies has been the uncertainty of the biologic activity of inorganic Cr and the lack of a purified, biologically active Cr compound. Consequently, a number of different types of Cr supplements have been used, including different strains and quantities of brewer's yeast, synthetic Cr compounds, and inorganic Cr^{3+}. Results of these studies have been variable, depending on which Cr supplement was used and in what quantity, the possible unrecognized effects of other nutrients in the yeast supplements, which subjects were studied, what subject variables affected the glucose response, and what methodologic procedures and measures were employed.

TARGET GROUPS

Malnourished children Whether or not inorganic Cr has been effective in improving glucose tolerance in malnourished children remains questionable. Of 19 malnourished Jordanian infants given a single oral dose of 250 μg of $CrCl_3$, six children had glucose disappearance values that improved (40). A similar study carried out in undernourished children in Egypt could show no effect of $CrCl_3$ on glucose tolerance (13), but improved glucose removal rates were reported for 14 marasmic Turkish children who received $CrCl_3$ (30). Since preexisting Cr deficiency states were not documented in these studies and other therapeutic interventions were being carried out, it is impossible to conclude that the Cr was responsible for the glucose tolerance improvement in those children who showed a change.

Elderly persons In studies in the 1960s and 1970s, improved glucose disposal rates were reported in about half of the elderly subjects given inorganic Cr or brewer's yeast supplements for periods of several weeks to four months (18, 39, 49, 74). More recent supplementation trials in middle-aged and elderly people might have been expected to provide more definitive data because they have been conducted with placebo groups, either single- or double-blind, with some in a crossover design. The data from the well over 200 participating individuals have been suggestive but far from conclusive.

Two groups of investigators found that glucose tolerances improved after they fed $CrCl_3$ or brewer's yeast supplements to elderly subjects deemed to be "at risk" (54, 65). However, in a study of 23 elderly free-living New Yorkers, mean age 73, who were given 5 g of brewer's yeast, or 200 μg of Cr as $CrCl_3$, or placebo, there were no changes in glucose, insulin, or lipid values (67). In retrospect, this latter group, though elderly, appears not to have been at risk of Cr deficiency, since careful 24-hour diet records showed that of nine nutrients calculated, only calcium was taken at less than 100% of the Recommended Dietary Allowances. Such an otherwise adequate diet might be expected to contain enough Cr to prevent deficiency. In groups of supplemented middle-aged and older adults, glucose tolerance improved little but some improvement in insulin levels was reported (52, 79).

Studies of the effect of Cr supplementation on lipid levels are complicated by the same array of factors that hamper glucose and insulin studies. Consequently, the results are unclear. In several studies, cholesterol decreased, high-density lipoprotein (HDL) cholesterol increased, and triglycerides were unaffected (22, 29, 52, 65, 79). But no effect on cholesterol levels was found in several other studies (7, 67, 75, 102). Until lipid changes can be correlated with accurately determined Cr levels in the body, no conclusions on the effect of Cr on lipids can be drawn.

Patients with diabetes mellitus Most of the earlier studies that suggested that Cr supplementation could improve glucose tolerance in diabetics were conducted without controls (18, 26, 27, 31, 52). Among more recent studies, conducted in a crossover double-blind design, one reported improved glucose tolerance in hyperglycemic subjects (7) while three others did not find improvement (75, 93, 102). A group of Chinese researchers recently reported favorable results for Cr supplementation in a double-blind study of 63 non-insulin-dependent diabetics (15). Blood glucose of patients who received a high-Cr yeast decreased significantly at fasting and at one-, two-, and three-hour time points after the administration of an oral dose of glucose. There were no changes in patients who received a low-Cr yeast or placebo. However, it must be kept in mind that the two yeasts differed not only in Cr content, but in other nutritional constituents as well.

Thus the possibility of abnormal Cr status in diabetes mellitus remains unproven. This is not surprising when we consider the heterogeneity of the diabetic population and the fact that there is no way of separating for study those diabetics whose diets might be Cr deficient from those who might suffer from impaired Cr absorption, faulty Cr conversion to the biologically active molecule, a metabolic defect, or excessive urinary excretion of Cr (47). Since all of these have been suggested as possible lesions, the effects of supplementation can be expected to vary among them. There is need for careful research using better methodology.

REASONS FOR INCONSISTENT RESULTS The inconsistent results of these studies may be due, in part, to the inability to select individuals who are clearly Cr deficient, to control for the many variables in dietary and other living habits, and to use supplements that are, themselves, comparable. The studies may have been done on the wrong target populations, so that supplementation was given either to subjects with normal glucose tolerance to begin with, and who were therefore unlikely to suffer from Cr deficiency, or to subjects who, while showing abnormal glucose tolerance, had normal Cr status and thus would not be expected to respond to supplementary Cr. Also, an adequate effect might not be obtained with inorganic Cr in individuals who might have problems absorbing or converting the inorganic Cr to the active form.

These studies illustrate the many problems in trying to reach definitive conclusions about the effects on metabolic function of brewer's yeast, reported to be the best food source of biologically active Cr (55). Yeast contains a great number of vitamins and minerals. It is impossible to feed such a material and be sure that a biological effect is produced by only one ingredient of the many that are ingested. It is for this reason that investigators have tried inorganic Cr salts. Also, yeast Cr cannot be compared gram for gram with

$CrCl_3$ because the two differ quantitatively with regard to Cr content. The amount of Cr in a gram of supplemental brewer's yeast is about one microgram. The commonly used daily dose of supplemental inorganic Cr is 200 μg as the $CrCl_3$ salt. If equal amounts of organic and inorganic Cr were to be compared, unacceptably large doses of yeast would be needed. However, if the Cr in brewer's yeast is handled differently than $CrCl_3$ in the body, as postulated, a lower dose of yeast Cr might produce a better physiologic response than a higher dose of $CrCl_3$. Thus, a standardized Cr supplement, purified from yeast or synthesized in the laboratory, is needed to resolve the disparate findings reported.

Cr Deficiency as a Factor in Other Conditions

In addition to malnutrition, pregnancy, aging, and diabetes, other metabolic stresses have also been monitored for possible links between Cr deficiency and status. These include the iron overload disease, hemochromatosis (80), and various forms of trauma (9) and stress (5). In hemochromatosis, the transport protein, transferrin, is saturated with iron (Fe). Cr has been shown to bind competitively to transferrin such that saturation, in vitro, with either Fe^{3+} or Cr^{3+} causes diminished binding of the other (41). When Sargent et al (80) injected $^{51}CrCl_3$ intravenously into six iron-loaded hemochromatosis patients and monitored the disappearance of the Cr tracer, they found that significantly less Cr was retained by these patients than by five normal and six iron-depleted patients. The investigators hypothesized that the diabetes associated with hemochromatosis is caused by the decreased binding and transport of Cr, which results in excess loss of Cr from the body. They postulated that this induces a Cr-deficient state at the tissue level and leads to the glucose intolerance of hemochromatosis. Whether the excess loss of Cr from the body in these patients is actually causally associated with hemochromatosis diabetes is not yet proven, but it is a most intriguing question to be pursued.

Because a transient diabetes is not uncommon during pregnancy, Wallach & Verch (112) followed the course of injected $^{51}Cr^{3+}$ in pregnant rats. Serum and tissue Cr decreased 50 to 80%, uterine Cr did not change, and almost 1% of the injected Cr was found in placenta; this suggests that maternal Cr may be depleted during pregnancy. No studies are available in humans.

MEASUREMENT OF Cr IN THE BODY

Since methods to measure Cr in body tissues and fluids have been inadequate until recently, the literature reporting these measures has been unreliable. Many questions remain regarding how the Cr in urine, serum, plasma, and hair relates to clinical changes caused by Cr depletion and repletion.

Cr in Blood

The use of plasma or serum to indicate Cr status is questionable. It has generally been dismissed (55) for two reasons. First, when tracer doses of ^{51}Cr were injected intravenously, they disappeared rapidly (57) rather than equilibrating with body tissues. Thus, it appeared that Cr in plasma or serum did not reflect body stores. Second, although plasma or serum Cr increases two- or threefold following supplementation with 200 μg of $CrCl_3$, these increases did not correlate with glucose or insulin levels in blood samples, whether from subjects in the fasted state or after a glucose load (3, 67). Earlier data had suggested that an increase in plasma Cr after glucose administration might be a reflection of Cr status (26, 49, 52). But those data, as well as some equivocal (33) and some contradictory earlier data (17, 73), cannot be evaluated because of the inadequacy of the analytical methods.

Other data suggest that serum Cr might yet prove of value in assessing Cr intake and status. The studies by Lim et al (51) on the distribution and kinetics of intravenously administered $^{51}Cr^{3+}$ in the body indicate a plasma pool in equilibrium with tissue compartments having fast (hours), medium (days), and slow (months) turnover rates. Recently, Randall & Gibson (77) found that serum Cr levels in tannery workers correlated positively with the ratios of urinary Cr to creatinine ratios. The levels of Cr in the exposed workers were comparable to levels in subjects receiving dietary Cr supplements, and the findings are consistent with Lim's kinetic studies. Also of interest is a finding by Earle et al (21) that obese subjects had higher plasma Cr (1.18 ± 0.71 ng/ml) levels following a glucose load than did lean subjects (0.71 ± 0.38 ng/ml) and that these levels correlated with insulin levels. They speculate that this might be due to the need to compensate for greater insulin resistance in the obese and diabetic subjects. These data are difficult to evaluate because, although the levels of Cr reported in the normal subjects are somewhat higher than the "normal" plasma values currently accepted (3, 50, 64, 104, 105, 110), the analyses were conducted under very well-controlled conditions, using methods validated with verified reference sera.

Cr in Urine

Measurements of Cr in the urine have not provided interpretable information about Cr status. In single urine samples, there are large random fluctuations in Cr, even when correction is made for urinary volume (6, 67). Twenty-four-hour urine collections are more reliable but they are not useful markers of Cr status because they fail to correlate with glucose, insulin, or lipid measurements (6, 67). However, urinary Cr measurements are useful for studying Cr absorption, retention, and excretion in balance studies (11, 68) and for

detecting conditions in which Cr losses may be abnormally high (9, 33, 47). The measurement of Cr in urine is also practical for monitoring the compliance of subjects who are given $CrCl_3$ supplements because their urinary Cr levels increase consistently and return to baseline levels after the supplement is discontinued (8, 67).

Cr in Hair

The potential advantages of using hair as an index of Cr status are that sample collection is noninvasive and that the levels of trace minerals in hair are considerably higher than in blood or urine. Investigators have reported Cr levels in hair in both normal and diabetic children and adults. Randall & Gibson (78) recently reported median hair Cr levels of 124 ng/g, ranging from 79 to 210 ng/g, in a group of 53 Canadian men. The determinations were made by neutron activation analysis, using validated methods of sample collection, preparation, and analysis and a reference hair powder (72). These men served as a control group for studying the exposure of tannery workers to Cr. The median concentration of Cr in the hair of the exposed tannery workers was 453 ng/g, ranging from 248 to 738 ng/g. These hair Cr levels correlated well with the higher serum levels in the exposed men, and thus, under well-controlled conditions such as those of this study, hair Cr may be a useful measure of body levels of Cr.

In contrast, earlier investigators reported hair Cr values that were one or more orders of magnitude higher. In a series of papers published between 1968 and 1974 and including carefully researched methodologic procedures, Hambidge et al (35–37) reported that average hair Cr was significantly lower in diabetic (560 μg) than in normal (850 μg) children and lower in parous (220 μg) than in nulliparous women (750 μg). Mahalko & Bennion (53) also reported significantly lower levels for parous than nulliparous women. But the difficulties of measuring and interpreting Cr levels in hair led Hambidge to question the usefulness of these measurements (34).

Rabinowitz et al (76) could not find statistically significant differences in hair Cr between a group of 46 diabetic men and a group of 20 nondiabetic men. But their concentrations are also nearly an order of magnitude higher than the measurements made by Randall & Gibson (78). In another study, lower (but still rather high) baseline levels of Cr in hair make it difficult to evaluate the finding that 39 diabetic subjects had hair Cr levels (317 $\pm$ 64 ppb) below those of 39 nondiabetics (383 $\pm$ 775 ppb) (42). Hair Cr levels before and after brewer's yeast supplementation could not be related to any improvement in diabetes but, because of inaccurate Cr measurements, it is impossible to evaluate this study.

Many variables are associated with measuring trace minerals in hair. These include age, sex, site of growth, hair color, hair treatment, water supplies,

binding of exogenous materials, and the removal of endogenous materials by chelating agents (96). Different physical properties of hair, variation in samples among subjects, seasonal variability in hair growth, and the effects of health and environmental conditions on hair growth patterns are all reasons why hair measurements are currently of little value in individuals (34). In summary, all of the earlier quantitative findings on hair Cr are suspect and their value is primarily to serve as pointers for future research.

Current and Future Capabilities

To elucidate the role of Cr in human nutrition, we must learn which tissues, fluids, or metabolites best reflect Cr status, what levels of Cr constitute deficiency, what molecular forms of Cr are important, what amounts of Cr in the diet are adequate, and how dietary Cr interacts with accompanying food constituents. A number of laboratories can now accurately measure Cr in picogram quantities in biological samples. Values of less than 0.2 μg/L in human serum, plasma and urine are now verified as normal (3, 50, 64, 68, 104, 108, 110). Highly sensitive and accurate instrumentation, dedicated "clean room" facilities to control environmental contamination, filtered air and water, ultrapure reagents, suitable reference materials (serum for measuring serum, urine for measuring urine) with which to verify accuracy, and trained technical personnel with "useful paranoia" (116) are essential to avoid contamination (104). Blood is drawn through siliconized needles into plastic, Cr-free syringes, and the preparation and analysis of samples and the interpretation of the data are critically evaluated.

Despite this progress in quantitation, a clinical measure of Cr status has eluded investigators. As we have pointed out, urine, hair, serum, and plasma have not fulfilled their earlier promise of becoming markers of choice. Red and white blood cells, the most readily available body tissues, are not now amenable to contamination-free methods of sample preparation for electrothermal atomic absorption spectrometry. Adipose tissue, a possible, although less accessible tissue, resists the available decomposition procedures needed to prepare it for analysis.

A parallel route for measuring the nutritional status of Cr is to clarify the nature and function of biologically active Cr. The early stages of this area of investigation were promising (55), but very limited progress has followed because the compounds in which Cr is active metabolically have not yet been determined. As a result, there is no way to be sure who is Cr deficient and it is consequently impossible to characterize accurately the role of Cr in human health and disease.

Cr METABOLISM AND FUNCTION

Cr is present in the diet in both the inorganic and the organically complexed form. Like other transition elements in the periodic table, Cr forms coordina-

tion compounds and chelates, which make it available for absorption and transport.

Absorption of Dietary Cr

The rate of Cr absorption ranges from <1% to 2% in animals and humans (4, 11, 68). Experimental evidence is lacking to support an early hypothesis (55) that glucose tolerance factor Cr is better absorbed than Cr^{3+} (16).

In the small intestine, Cr, like other trace elements, is subject to chemical interactions with dietary (exogenous) factors and with intestinal (endogenous) factors that may affect its absorption and assimilation in various ways. Some substances, such as amino acids (57), chelate Cr in the small intestine; in so doing they prevent Cr from precipitating at the alkaline pH of the intestine and enhance Cr absorption. Other small chelating agents may form Cr complexes that vary in their effects on absorption. Phytate significantly decreases Cr absorption across the rat intestine, both in vivo and in vitro (14). But oxalate, which is widely distributed in vegetables, plants, and feeds, significantly increases Cr transport. Also, an inverse relationship between dietary Cr absorption and iron status was reported by Gonzalez-Vergara et al (28), who found that Cr absorption was higher in iron-deficient mice than in iron-replete animals.

Other dietary components may either increase the need for Cr or act synergistically with Cr. When diets high in simple sugars were fed, the excretion of Cr in the urine increased from 10% to 300% for 27 of 37 subjects (8, 46). Conversely, glucose tolerance improved in four subjects fed a supplement containing Cr with nicotinic acid (101). It did not improve in eight other subjects who were given either Cr only or nicotinic acid only. The investigators postulate that nicotinic acid, rather than nicotinamide, the more common dietary form of niacin, is needed as a substrate for the synthesis of the biologically active Cr molecule. This finding would be of interest if it were confirmed in controlled experiments. A second synergistic effect is suggested for Cr and ascorbic acid by an experiment with guinea pigs (114) in which a combined Cr and ascorbic acid deficiency resulted in impaired glucose tolerance and elevated plasma cholesterol. Recent data by Dowling et al (20) have shown that Cr absorption is influenced by the plasma proteins, transferrin and albumin, as is the case for Fe and Zn (94). Much work remains to understand the web of effects that exogenous and endogenous constitutents may have on Cr absorption and to relate the chemical form of the dietary Cr to the absorptive process.

Cr Transport

Both transferrin and albumin may be capable of binding newly absorbed Cr and transporting it in the circulation (20). Transferrin was identified in 1964 as the plasma transport protein of Cr (41), and its saturation with Fe can result

in reduced transport and retention of Cr (80). Recent work by Sayato et al (81) and Yamamoto et al (119) verifies that trivalent Cr possesses a high binding activity for transferrin in plasma. Yamamoto et al (119) reported a low-molecular-weight Cr complex with a high affinity for trivalent Cr that demonstrated a significant, reversible transfer of Cr to transferrin but not to albumin. It may be that both transferrin and albumin influence Cr absorption, transferrin by binding newly absorbed Cr and albumin by assuming the role of acceptor and transporter of Cr if the transferrin binding sites are unavailable. In addition, other plasma protein fractions, including gamma- and beta-globulins, and lipoproteins bind Cr and may have a role in Cr metabolism.

Identification of Metabolically Active Molecules

Although there is ample evidence that Cr exists in biological systems in the form of organically bound compounds, a single, specific organic Cr compound that is biologically active has not been characterized. Earlier studies postulated such a molecule consisting of Cr, nicotinic acid, and the amino acids glutamic acid, cysteine, and glycine (55, 61, 99). But disparate findings have resulted from some of the recent studies.

Keinle et al (45) attempted to synthesize glucose tolerance factor (GTF) but were unable to purify a crude extract that contained two GTF-like chromium coordinated compounds besides all of the starting materials. Although their extract exhibited GTF-like activity, it did not appear to be involved in the interaction of insulin with its membrane receptor. Yamamoto et al (118) recently isolated a low-molecular-weight Cr-binding substance from mouse liver, kidney, plasma, erythrocytes, urine, and feces following an intraperitoneal injection of hexavalent Cr in the form of potassium dichromate ($K_2Cr_2O_7$). The low-molecular-weight Cr-binding species contained trivalent Cr, amino acids, and some other nonidentified UV-absorbable components and could be separated into two subfractions with molecular weights of 1600 and 2600. It is thought to exist originally in organ-cytosol in a Cr-free apo form, which then finds Cr as required. Yamamoto postulated that this substance plays an important role in the incorporation of Cr into liver cells and in the release of Cr to the circulation, urine, and feces.

Mirsky et al (61, 62) were able to synthesize a molecule with good activity in a yeast cell system, but Haylock and coworkers (38) found that, upon separation and purification from brewer's yeast cells, their biologically active "glucose tolerance factor" did not even contain Cr. Their results and work by Hwang et al (43) suggested that glucose tolerance factor from brewer's yeast cannot be regarded as a Cr complex.

In contrast, Gonzalez-Vergara et al (28) synthesized a series of ^{51}Cr coordination complexes with Schiff bases. These Cr Schiff-base chelates were absorbed and they accumulated in liver at higher concentrations than un-

complexed Cr. Simple GTF-model complexes showed almost no accumulation by liver, although they and uncomplexed Cr were better retained by the body than the chelates. Since the chelates were particularly stable when coordinated with nicotinic acid, these investigators postulated that the nicotinic acid may affect biodistribution rather than affecting absorption or utilization. Finally, they suggest that Cr chelates may be nutritionally relevant and that naturally occurring GTF complexes containing nicotinic acid may not be unique in this respect.

In summary, it is not unlikely that there may be molecules that do not contain Cr but do enhance insulin action. It is also not unlikely that there may be more than one Cr-containing, biologically active molecule. Thus, the designation "biologically active Cr" may be a more apt descriptor than the currently ill-defined "glucose tolerance factor."

Other Metabolic Functions

Cr may not only increase the transport of sugars into cells by binding to insulin (23, 55, 62), but may also have a role in RNA synthesis. Recently, a Japanese group administered 0.005 to 5 mg/kg of $^{51}CrCl_3$ to mice and found that about 20% of the dose was retained in the nuclei of liver and that RNA synthesis was enhanced (70). The effect of the Cr^{3+} was dose dependent and the use of Cr^{6+} was inhibitory. In a second experiment, they investigated hepatic nucleolar RNA synthesis in partially hepatectomized rats (71). Synthesis was enhanced and the RNA proceeded to rRNA. To test their hypothesis that Cr, accumulated in nucleoli, may participate in nucleolar gene expression, they studied a chromatin-Cr complex prepared from mouse liver chromatin and $CrCl_3$ (69). The results of this in vitro experiment suggested that Cr^{3+} was bound preferentially to DNA in chromatin and caused an increase in initiation sites, thus enhancing RNA synthesis.

IS Cr MARGINAL OR INADEQUATE IN THE DIETS OF SOME PEOPLE?

Cr levels well below the 50 to 200 μg/day that are suggested in the 1980 RDA have recently been measured in diets of healthy adults varying in age, geographic, ethnic, and socioeconomic status (4, 11, 25, 67, 68). Diets consumed by two different groups of free-living, well-nourished elderly volunteers contained an average of 37 μg/day (range 15 to 55 μg) (67) and 25 μg/day (range 14 to 48 μg) (11). Most of these subjects were ingesting otherwise nutritionally adequate diets. These Cr levels were similar to the 39 μg/day (range 11 to 84 μg) measured in the diets of a group of healthy young adults (67). In 28 American diets containing 2800 kcal and 43% fat, Cr averaged 62 μg/day and ranged from 37 to 130 μg/day. When fat was 25%

of the calories, Cr was somewhat higher, averaging 89 μg/d and ranging from 41 to 224 μg/day (48). Expressed per 1000 kcal, Cr in the diets of most of the volunteer subjects was between 20 and 25 μg/1000 kcal. Average Cr content was 22 μg/1000 kcal in the 2800-kcal diets containing 43% fat and 32 μg/1000 kcal in the diets containing 25% fat.

Evidence from balance studies conducted in two men on a metabolic ward (66) raises the question of whether such levels are consistent with positive metabolic balance under varying conditions. In these men, a daily dietary Cr intake of 37 μg was accompanied by fecal Cr excretion of 36 μg/d and urinary Cr losses of 0.29 μg/d. While the subjects were in apparent equilibrium, dermal and hair losses were not measured, and the data must be interpreted cautiously because of the analytical problems. Concurring data were reported by Bunker et al (11) for a five-day balance study in free-living subjects. If, as found by Kozlovsky et al (46), high dietary levels of simple sugars cause increased Cr losses via the urine, Cr balance could be compromised if dietary Cr is marginal.

These data suggest several possibilities: (*a*) that dietary Cr levels below 50 μg are, indeed, marginal and that metabolic penalities may result, or (*b*) that the quality of the dietary Cr (inorganic vs organically complexed) really does matter, or (*c*) that human Cr requirements are not as high as previously thought in healthy individuals. A fourth possibility is that such intakes may be too low to maintain Cr balance if dietary factors that inhibit Cr absorption are present (12), or when illness (9, 10, 24, 30, 42, 44, 80), aging (113), or other stresses (5, 13, 25, 33, 35–37, 46, 73, 112, 115) increase Cr needs or losses. Thus, it is not now known whether these customary dietary Cr intakes are precarious in segments of the population.

In conclusion, whether dietary Cr deficiency is prevalent in some segments of the population remains an open question in nutrition research. If it is, what dietary and physiologic factors are involved? The resolution of these questions awaits a reliable measure of Cr, or of a Cr metabolite, in one or more body tissues or fluids and the correlation of this measure with one or more physiologic functions as well as with the type and quantity of Cr in the diet.

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