Interaction of Chromium with Insulin: A Progress Report

Recent studies have contributed to the knowledge of the interaction of chromium with insulin and the possible implications for carbohydrate and lipid metabolism.

There is evidence for a biologic role of trivalent chromium in three areas of metabolism. First, at least three groups of investigators have suggested an interaction of chromium with thyroid function in human or animal studies,[1–3] but neither the mechanism nor the physiologic significance of this interaction is known. Second, early studies by Wacker and Vallee[4,5] detected very high chromium concentrations of great stability in various liver fractions containing nucleic acids and protection by the element against heat-induced conformational change. These findings, which suggest a function of chromium in nucleic acid metabolism, were followed by the demonstration of a nearly threefold stimulation of amino acid incorporation into protein of isolated liver nuclei by chromium, much greater than that of any other metals tested.[6] During the 1980s, Okada et al.[7] provided evidence for a direct interaction of chromium with DNA templates that resulted in a significant stimulation of RNA synthesis in vitro. Further work by these investigators identified a protein of approximately 70 kDa that contained 5–6 atoms of chromium in regenerating liver and ascribed to it the stimulation previously found in vitro.[8] No follow-up studies to this important work are known to this reviewer. Third, the nearly 40 years of work on the interaction of chromium with insulin have produced much knowledge of its physiologic role and two basic, unanswered questions as to its mechanism of action and its active form. The studies reviewed here[9,10] have furnished some important answers.

Two years after the findings establishing a chromium requirement for maintenance of normal glucose tolerance in rats, in vitro studies with epididymal adipose tissue from chromium-deficient rats demonstrated a mild but significant potentiation of the insulin action on glucose oxidation by inorganic chromium complexes, resulting in a greater slope of the dose-response curve to insulin.[11] Subsequently, measuring the cell transport of the nonutilizable sugar D-galactose gave the same results,[12] which led to the conclusion that the site of chromium's action and interaction was at the first step of metabolism, cell transport.

Similar potentiation of insulin was reported in other insulin-responsive systems. The rate of insulin-induced swelling of isolated liver mitochondria was significantly enhanced by the addition of chromium,[13] as was the insulin-stimulated glucose uptake of isolated lenses of low-chromium rats.[14] The effect of the in vitro addition of insulin on the respiratory quotient of epididymal adipose tissue was significantly enhanced by chromium, as shown not only by an increased slope of the dose-response but also by a marked shortening of the lag phase.[15] Polarographic studies of mitochondria suggesting the participation of chromium in a ternary complex between insulin and membrane sulfhydryl groups[16] were not further pursued, in part because of the known low tendency of chromium to coordinate with sulfur. The results of in vivo studies on hypoglycemic, glycogenic action of insulin, on amino acid transport, and on incorporation of insulin-sensitive amino acids into protein confirmed the potentiating effect of chromium on insulin of the in vitro studies,[17] but provided no information on the exact mechanism of that interaction. This conclusion remained valid during the subsequent decades of chromium research until 1996, when Davis et al.[9] discovered an activation of a phosphotyrosine phosphatase (PTP) in isolated fat cell membranes of rats.

The assay consisted of membrane fragments obtained from adipocytes of chromium-adequate rats as a source of PTP, with p-nitrophenyl phosphate (5 mM) in tris buffer at pH 7.5 as substrate and a low-molecular-weight chromium-binding substance (LMWCr) of approximately 1500 Da, containing four chromium atoms per molecule. The reaction was terminated after 1 hour at 37 °C and optical density of the product measured at 404 nm. Although the LMWCr compound did not affect the reaction in the absence of membrane fragments, it stimulated PTP activity significantly in the complete system in a dose-dependent fashion when concentrations representing 0.1–10 nM chromium were added. Increasing the addition beyond that concentration did not further increase the maximal response of about 1.5-fold over controls. Additional studies with phosphoserine-containing substrates and the use of a kinase inhibitor strongly suggested specificity of the effect for PTP.

The observation that the addition of chromic chloride in the absence of the low-molecular-weight apo preparation was ineffective emphasizes the importance of the organic coordination of the element. Titration of the apo compound with inorganic chromium and subsequent activity determination of the reaction products established a sharp break in the dose-response curve at four atoms of chromium per molecule, after which activity increased only insignificantly. Substituting other transition metals for chromium resulted in inhibition of the activity, except for iron, which produced half the effect of chromium, and a

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minor, insignificant stimulation by manganese.

These results have provided challenging new ideas as to the mode and site of the actions of chromium, but they also present some unexplained problems. One relates to the slow ligand exchange rate of chromium, which argues against the direct participation of the metal in enzyme reactions. The other, more important problem is the lack of any need for or interaction with insulin, in contrast to practically all known information on chromium-responsive systems.

The authors' subsequent studies of isolated insulin receptors and their phosphorylating/dephosphorylating reactions, a logical extension of their previous work, does not suffer from these problems.[10] The system consisted of isolated fat cell membranes or of rat liver insulin receptors, in which the activity of protein phosphotyrosine kinase (PTK) was measured after 75 minutes of incubation at 37 °C in the presence or absence of the LMWCr. In contrast to the results with PTP discussed above, the new system using PTK required the presence of insulin to elicit an effect of LMWCr, regardless of whether cell membranes or insulin receptors were assayed. Insulin at 100 nM concentration was incubated with insulin receptors for 2 hours at pH 7.4 and 4 °C prior to the PTK assay.

In the absence of LMWCr, 100 nM insulin stimulated the enzyme activity by about 60%. Addition of the low-molecular-weight (LMW) apo compound produced a small additional effect, probably owing to some residual chromium in the preparation, whereas the complete LMWCr (4 Cr atoms/molecule) resulted in a sevenfold stimulation of the activity. Addition of antibodies to the insulin receptor abolished the effect. As in the previous report, the question of the specificity of the effect was investigated by reacting LMW apo with other transition metals (vanadium, manganese, iron, cobalt, nickel, copper, zinc, molybdenum) and assaying the activity of the products on membrane PTK activity. None of these metals were active, whether added to the system separately or as preformed metal-LMWapo complexes. These results are consistent with the known dependence of the chromium effect on insulin, and they clearly establish the insulin receptors as the site of action in the regulation of carbohydrate metabolism. They must be considered among the most important contributions to the field.

There is, however, one very important question left unanswered: What is the chromium-containing compound that produces the results described in these two publications? The authors referred to Yamamoto's method of preparation and to his conclusions about the chemical composition of the compound.[18] They also report the isolation of substantial quantities of the compound from bovine liver and partial characterization of tetranuclear (or two dinuclear) chromium assemblies in the oligopeptide.[19] In contrast to Yamamoto's statements, they discount the previous work on the glucose tolerance factor[20] by stating that "the latter has no intrinsic functions in mammals."[9] They also fail to mention other recent, productive work on a biologically active form of chromium.[21] It is appropriate, therefore, to summarize briefly the present state of knowledge of the active form of chromium.

The existence of a "glucose tolerance factor" (GTF) was postulated in 1957 with the chemical separation of a substance in brewer's yeast and pork kidney powder that prevented the age-related decline of intravenous glucose tolerance in rats from an agent called Factor 3 that protected rats against dietary necrotic liver degeneration.[22] When, 2 years later, trivalent chromium was identified as the active ingredient of GTF, the examination in vivo and in vitro of many known chromium compounds revealed marked differences between the activity of that category and purified extracts from natural sources. Subsequent efforts to further purify and identify the active compound(s) demonstrated that a compound consisting of chromium coordinated to two nicotinic acid molecules and to the amino acid constituents of glutathione possessed GTF activity.[20] Synthetic preparations of that composition closely resembled the chemical behavior and biologic activity of the natural product,[20,23] but further attempts to isolate and crystallize the substance failed and the project was abandoned.

Several attempts by others to synthesize chromium compounds with GTF activity or to identify the structure of active extracts from natural sources were reviewed in 1985.[24] Two groups are known to this reviewer to be still active in this field. Mirsky et al.[25] pursued the original work of Burkeholder and Mertz[26] on the production and effect of a substance with GTF activity by yeast and, by 1993, reported on purified yeast extracts that significantly reduced blood glucose and free fatty acids of steptozotocin diabetic rats.[27] Further results of their work on the nature and biologic action of the substance can be expected. The second group attempting to purify and isolate a substance with GTF activity and define its mechanism of action is the main subject of this review.[9,10]

The discovery of that substance, LMWCr, goes back to Wu and Wada,[28] who in 1981 purified a chromium-containing compound from rat liver. The compound, which could also be found in other organs and urine, bound trivalent chromium in vitro and contained the element after intravenous injection of chromate into the donor animals. It was stable against boiling for 10 minutes, similar to a liver extract with biologic activity on glucose metabolism described earlier.[29] What first appeared to be mainly a detoxification function (reduction and excretion of toxic hexavalent compounds)[30] was soon expanded into an important physiologic action: the LMWCr purified from rabbit liver enhanced glucose transport and oxidation by epididymal fat tissue in the presence of insulin at nearly physi-

ologic chromium concentrations.[31] The compound had a molecular weight of approximately 1500; amino acid analysis revealed the constituents of glutathione, aspartic acid, and chromium at a ratio of four terminal amino residues per chromium molecule. The authors also reported a specific absorption peak at λ_{max}=260 nm, similar to that of the yeast-derived GTF.[20]

Their subsequent discovery of bovine colostrum as a reliable source of LMWCr compounds[31] has special physiologic significance. It suggests a requirement of the newborn organism for detoxification or, more likely, as a source of bioavailable chromium and gives information on the interaction between chromium and its LMW apo compound. Compared with properties of liver-derived LMWCr, the active fraction from colostrum, which represents about one-third of the chromium, had a molecular weight of 1500, the same ultraviolet absorption, the same amino acid composition, and the same biologic activity in vitro. It differed, however, in the ratio of chromium to the amino terminals, 0.25:1 compared with 4:1, for the liver-derived compound. This difference is explained by the fact that the donor animals of the latter, but not those of the colostrum, were injected with chromium, suggesting that LMWCr is not normally saturated with chromium and has a large potential to bind and detoxify excesses. This is the latest publication on this important subject by these authors known to this reviewer, but their work is being continued and extended by Davis and Vincent.[9,10,19] The exact structure of LMWCr or of any other substance with GTF activity is still unknown.

In light of the new information on the site and mode of action of LMWCr discussed above,[10] it appears desirable to discuss and possibly reconcile the information on the nature of the substance(s) with GTF activity proposed independently by the three groups. The first mention of GTF in the literature carried the title "A Glucose Tolerance Factor and Its Differentiation from Factor 3,"[22] which clearly indicated the probability that more such factors with similar biologic activities may exist. The same authors described one such agent with slightly different biologic properties a few years later,[29] and Yamamoto emphasized the occurrence of different forms of LMWCr.[32] Mirsky's[21] GTF may be slightly different in its biologic properties from the GTF described by Toepfer et al.[20]

At this time, given the lack of information on the exact structure of the compounds and their physiologic behavior, any comparison of their potential importance would not be productive. More productive would be studies of the in vivo effects of the compounds now available. GTF was so named because of its effect on intravenous glucose tolerance in the intact organism. In that form, chromium was much more available to the fetus of rats than were inorganic compounds,[33,34] and there is some evidence that the increase (or decrease) of circulating chromium in response to a glucose load represents GTF. Investigation of these areas will provide much-needed information, complementing the new knowledge of the interaction of chromium with the insulin receptor.

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Fish Consumption and Risk of Sudden Cardiac Death

Studies show conflicting results regarding the protective effect of dietary fish and fish oil on certain types of cardiovascular disease. A recent epidemiologic study supports the hypothesis that moderate consumption (1–2 meals/week) of fish lowers the risk of sudden cardiac death in humans.

Omega-3 (ω-3) fatty acids have many physiologic effects, several of which are known to influence cardiovascular disease risk, including lowering plasma triglycerides, inhibiting plaque formation, decreasing platelet aggregation, and altering arrhythmogenesis.[1] Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the biologically active ω-3 fatty acids, can be synthesized in the body from α-linolenic acid, which is found principally in canola and soybean oils and walnuts. EPA and DHA are also found in the diet, predominantly in fatty fish (Table 1). Dietary intake of these fatty acids is strongly correlated with their concentration in plasma, red blood cell membranes, and platelets.[2–4]

In the early 1970s, Bang et al.[5] made the observation that, despite higher dietary fat intakes, Greenland Eskimos exhibited significantly lower rates of mortality from coronary heart disease compared with Danes. The researchers suggested that this finding might be attributed to differences in the consumption of seafood, because Eskimos have higher intakes of fatty fish, which are rich sources of ω-3 fatty acids. In a prospective cohort study, Kromhout et al.[6] also demonstrated that consumption of fish once or twice a week was associated with a 50% decline in mortality from coronary heart disease. Many other studies have reported similar protective effects of fish consumption.[7] More recently, however, several cohort studies have found no effect of fish consumption on coronary heart disease.[8,9] Variability in findings may be due, in part, to the differences in choice of endpoint (i.e., overall mortality versus mortality from coronary heart disease, sudden cardiac death versus nonsudden death, primary

Table 1. Omega-3 Fatty Acid Composition of Selected Types of Fish

Fish Type	g/100 g
Dark fish[a]	1.37
Tuna	0.69
Shellfish	0.46
Other fish	0.17

[a]Salmon, bluefish, mackerel, sardines, swordfish.

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