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Role of chromium supplementation in Indians with type 2 diabetes mellitus

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Abstract

Type 2 diabetes mellitus is a complex metabolic disorder with adverse cardiovascular risk. The role of micronutrients has not yet been well clarified in this condition, especially in India.

The objectives of this study were to: (1) evaluate chromium status in Indian subjects with type 2 diabetes mellitus, (2) assess the effect of chromium picolinate (200 μg trivalent chromium twice daily) administration on glycaemic control and lipid profile in these subjects and (3) comment on the possible mechanism of any beneficial effect noted above.

Fifty subjects were studied in a double blind, placebo-controlled, crossover fashion, with each treatment arm (chromium/placebo) lasting 12 weeks and 4 weeks' wash-off period in between. 50 healthy age- and sex-matched volunteers served as controls. Serum chromium level appeared to be higher in the general population in our country compared to western countries (36.5–59.5 nmol/L as compared to 2.3–40.3 nmol/L) However, the local diabetics were found to have a lower serum chromium level than the healthy controls (32.3 nmol/L against 44.7 nmol/L; $p < 0.0001$) and a mean increase of 3.5 nmol/L was noted after 12 weeks of chromium supplementation that was, expectedly, not seen in the placebo phase ($p < 0.0001$).

Significant improvement in glycaemic control was noted in the chromium-treated group (ΔFasting serum glucose = 0.44 mmol/L, $p < 0.001$; ΔPost-prandial serum glucose = 1.97 mmol/L, $p < 0.001$; Δglycated hemoglobin = 0.01; $p = 0.04$, in comparison to placebo) This was accompanied by a significant greater fall in fasting serum insulin in the chromium-treated group, $p < 0.05$.

The change in lipid parameters (total serum cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and triglycerides) did not show significant difference between the chromium and placebo groups.

Clinically significant hematological, renal or hepatic toxicity were excluded by routine hemogram, serum urea, creatinine, alanine amino transferase (ALT) and alkaline phosphatase estimations.

In conclusion, chromium supplementation seems to improve glycaemic control in type 2 diabetic patients, which appears to be due to an increase in insulin action rather than stimulation of insulin secretion.

Keywords: Chromium; Picolinate; Glycaemic control; Lipid parameters

1. Introduction

Diabetes mellitus is a very common metabolic disorder with the potential to cause devastating chronic complications. In this entity, almost every facet of metabolism, including the metabolism of micronutrients, is affected. Chromium (Cr) is one such micronutrient, whose role in carbohydrate metabolism and in insulin action still remains undefined, despite a quarter of a century of relevant research.

Chromium, a lustrous metallic element mainly used in glass and alloy industries, has 3 valences—II, III and VI [1]. It is a nutritionally essential element with a requirement in humans of 0.005–0.2 mg/day and serum level of 2.3–40.3 nmol/L [1,2]. It is the trivalent Chromium Cr_3 that has been studied extensively [3]. It is the most stable form which exists as soluble and insoluble salts as well as complexed

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with organic ligands eg. as Glucose Tolerance Factor (GTF), in yeast and as Low Molecular Weight Chromium-like substance (LMWCr) in animal cells. It is slowly absorbed, binds to DNA and resides in nucleus in association with chromatin [4]. Following prolonged use of Cr_3 in dosage 350 times more than the Reference dose set by the United States Environmental Protection Agency (EPA) [5], no significant harmful effects have been observed. With increased level i.e. >20 μg/ml, oxidative stress, reduction of cytochrome-C and DNA breaks may be produced [6].

The hexavalent form of Cr is recognized as toxic in industrial exposures which can result in asthma and bronchitis in short term [7] and is carcinogenic to skin and respiratory tract upon long term exposure [8]. Its cell penetration is 1000 times more than Cr_3; it enters erythrocytes and binds to globin fraction of hemoglobin where it is oxidized to Cr_3. The bivalent form is a strong reducing agent easily oxidized to Cr_3. It has been postulated that intracellular Cr_3 can be reduced to Cr_2 by L-cysteine and NADH which in turn generates toxic OH^- radical [9].

Chromium possibly influences glucose metabolism by helping in the binding of insulin to its receptors and potentiating its action [10]. Insulin resistance, which is central to the pathogenesis of type 2 diabetes mellitus [11], may be a consequence of Chromium deficiency. Attempts have been made to identify Chromium deficiency in diabetes and to supplement dietary Chromium to ameliorate symptoms in diabetics [12]. Therapeutic trials with trivalent Chromium supplementation have produced equivocal results. In 1977 [13] severe diabetic symptoms of a female patient on total parenteral nutrition were alleviated by supplemental Chromium, along with reduction in exogenous insulin requirement. Similar results have been documented in three other studies [14,15,16]. Improvements in glucose and/or lipid concentration following Chromium supplementation have also been reported in children with protein calorie malnutrition [17], the elderly [18], individuals with type 2 diabetes mellitus [19] and impaired glucose tolerance [20,21]. Again, hypercholesterolaemia, an important link in the genesis of coronary artery disease [22] and aortic plaques, occurred in animal studies with diets deficient in Chromium [23] and showed regression on introduction of Chromium in the diet [24]. Supplemental Chromium to diabetic men has been reported to lead to significant improvement in glucose tolerance with lowered fasting glucose, plasma total cholesterol (Tc), LDL cholesterol (LDLc) and increased HDL cholesterol (HDLc) [25]. A significant reduction in plasma triglyceride (TG) has been reported in type 2 diabetes mellitus patients treated with Chromium [26].

The search for Cr-containing biologically active substances, since 1950, has identified several products which have all been termed GTF, composed of chromic ion, nicotinic acid and aminoacids glycine, glutamic acid and cysteine [27]. This agent, which potentiates the action of insulin, had been isolated initially from brewer's yeast and kidney powder [28]. It is an organic, low molecular weight complex containing trivalent Chromium, the exact structure, site and pathway of action of which are yet unknown.

In all the studies related to the action of Chromium on glucose/lipid metabolism, it has been seen that those who fail to get positive result, use inorganic Chromium [26], suggesting limited intestinal absorption and intracellular uptake of inorganic Chromium. Low serum Chromium levels are caused by inability to obtain enough Chromium from the diet, probably due to the obvious lack of the metal in diet or due to increased excretion following infection, pregnancy, high glucose diet and stress [29,30,31]. The availability of Chromium from the diet is also hampered by competing ions such as Cu^{++}, $Fe^{++,}$ Mn^{++}, and Zn^{++}. Identification of nicotinic acid, which is 3-carboxypyridine, as a component of GTF stimulated studies with other carboxypyridines like 2-carboxypyridine (picolinic acid) and 4-carboxypyridine (isonicotinic acid) [32,33]. Because of better absorbability of chromium picolinate [34], picolinic acid has been approved as a chelator for improved utilization of Chromium. This is synthesized from Tryptophan in kidney cells and brewer's yeast [18,35] and is also present in intestinal cells [36] and human milk [37]. Thus, picolinic acid may be a naturally produced ligand that facilitates the absorption and transport of ions in children and adults [38] and chromium picolinate has become a popular nutrient as well as therapeutic agent for adult-onset-diabetes mellitus [34].

Subsequently, a naturally occurring oligopeptide, low-molecular-weight Cr-binding substance, LMWCr [39,40], which is widely distributed in liver, kidney, spleen, intestines, testicles and brain [41] and binds four equivalents of Cr in a multicentric assembly, has been proposed [40,42]. It comprises amino acids glycine, cysteine, glutamic acid and aspartic acid. The chromic centers are bridged by anionic ligands and are supported by carboxylate group from aspartate and glutamate residues [40,43]. This LMWCr has been postulated to be a part of an insulin signal amplification mechanism [44] in which its apo-form, present in cells, is activated by binding Cr ions and mobilizing them into the cells. This binding results in stabilization of the active conformation of insulin receptor tyrosine kinase [45]. Due to its similarity to Calmodulin in structure and function, it has been named Chromodulin [46].

In 1997, a synthetic multinuclear chromic assembly $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_6]^-$ or Compound I, was found to mimic the insulin receptor kinase stimulating action of LMWCr [47]. In contrast to LMWCr which is readily excreted and apparently does not enter cells to an appreciable degree, this functional biomimetic has striking effect on plasma triglycerides, Tc, HDLc and LDLc after 12 weeks of supplementation in rats at a level of 20 μg/Kg body mass and may affect body weight and fat content [48]. No toxic effects or DNA damage (postulated following high doses of Cr picolinate) have been reported [49]. This closely approximates structural, spectroscopic and functional properties of LMWCr.

No definitive studies on human Chromium deficiency have been carried out, particularly in India, largely because of analytical difficulties in determining ultra-trace Chromium levels in tissues. This study aims to determine the Chromium status of type 2 diabetics and the therapeutic value of Chromium supplementation in these individuals.

2. Methods and materials

Subjects for this study were selected from patients attending the Diabetes clinic, S.S.K.M Hospital, Calcutta. They were 50 type 2 diabetic patients on diet alone or diet and oral hypoglycaemic agents with reasonably stable (not optimum in all cases) glycaemic control over the previous 3 months as determined by fasting plasma glucose and glycated hemoglobin values. Patients who were pregnant or with known allergy to chromium picolinate or already on multi-mineral supplementation were not included. Patients with chronic diabetic complications beyond background retinopathy, asymptomatic proteinuria, asymptomatic peripheral neuropathy or chronic stable angina were excluded. Likely need for additional medications that may influence insulin sensitivity (e.g. Beta-blocker, Thiazide, Glucocorticoids, ACE inhibitors) during the study or need for initiation of insulin therapy during the study were further criteria for non-inclusion. Friends and relatives volunteered as normal controls. Informed consent was obtained and the participants were carefully instructed not to change their diet and living habits during the trial including the wash out period.

Each patient was clinically evaluated at the initial visit; this included ophthalmic examination and record of current medications. Each patient was given a 5 weeks' supply of study medication/placebo at each visit, to be taken as one capsule twice daily. They were followed up every 4 weeks ($\pm$1 week) up to 28 weeks with a double-blind crossover after a 4 weeks' wash-off period after 12 weeks. Fasting (after 12 hr fast) blood samples were collected at 0, 12, 16 and 28 weeks for each patient for assay of glucose, glycated hemoglobin (HbA1c), insulin, chromium and lipid profile (cholesterol, LDLc, HDLc, triglyceride); safety monitoring was performed with the help of hemogram, creatinine, urea, ALT and alkaline phosphatase. During collection and preparation of samples, standard precautions to avoid hemolysis were observed, since damage to erythrocytes leads to release of intracellular chromium into the serum; thereby giving false high values.(Cr content of erythrocytes (3.84–69.2 nmol/L) is higher than the serum Cr value (2.3–40.3 nmol/L) [50]. Anti-hyperglycaemic medications and doses were unaltered, except in case of hypoglycemia. Glucose was assayed in fresh serum by the Glucose Oxidase method. For other analytes, serum samples were kept at -20°C (for up to 1 month) until analyzed. Serum chromium was measured by Atomic Absorption Spectrophotometer, coupled with graphite furnace (AA-SCAN-1, THERMOJERREL, ASH, USA) [51,52]. During standardization of this method, serum was initially directly injected into the graphite tube, but responses were abnormal in nature. Finally, this method was modified as per our available infrastructural facilities. Initial preconcentration of samples by using concentrated nitric acid mixed with hydrogen peroxide was followed by ashing at temperatures gradually elevated to 700°C. This ash was extracted by strong mineral acids in aqueous medium and the requisite volume was made up in a volumetric flask. Known volumes of samples in micro ml ranges were injected into the graphite tube. (Gradual increase in temperature was programmed from 25°C to 3000°C) The absorbance of the unknown samples were compared with known reference chromium standard, obtained from E. Merck, Germany. This very low level of chromium, present in the unknown samples was subtracted from the absorbance of standard containing samples. Moreover background corrections were made using D_2 lamp at the particular wave length of the Cr-max (λ357.9 nm) The complex matrix containing high organic matter was minimized in the above mentioned two steps.

Utmost care was taken for Chromium samples to be free from all standard contaminants encountered during chromium analysis, starting from quality of water to all containers. Glycated hemoglobin was measured by affinity chromatography [53]. Total cholesterol and triglyceride were estimated by enzymatic methods [54,55] while HDLc and LDLc were measured by utilizing precipitation reactions [56,57]. Serum creatinine, urea, alanine amino-transferase and alkaline phosphatase were estimated by standard biochemical methods [58,59,60,61]. Statistical analysis was done with the help of a statistical software package (SPSS PC + 4.0) The Kolmogorov Smirnov test (non-parametric test) was applied to examine for significant change of the variables between 2 groups of treatment. One-way analysis of variance was applied to compare the mean difference among the 3 groups (Chromium, placebo and normal) and where significant differences were present, multiple comparison test (Schaffe's test) was used to identify the significant difference of mean between the 2 groups.

Table 1
Comparison of clinical parameters of normal controls and Type-2 diabetic subjects

	Normal controls (Mean $\pm$ SD)	Type-2 DM subjects (Mean $\pm$ SD)	p-value
Age (years)	52.8 $\pm$ 11.5	53.5 $\pm$ 10.9	NS
Sex ratio (M:F)	3:2	1.9:1	—
BMI (Kg/m2)	21.8 $\pm$ 2.4	22.0 $\pm$ 3.1	$<$0.05
WHR	0.84 $\pm$ 0.1	0.93 $\pm$ 0.1	$<$0.05
Systolic blood pressure (mm of Hg)	119.2 $\pm$ 5.4	133.3 $\pm$ 17.8	$<$0.0001
Diastolic blood pressure (mm of Hg)	77.2 $\pm$ 4.9	85.7 $\pm$ 8.8	$<$0.0001

NS: Not significant

Table 2
Comparison of biochemical parameters of normal controls and Type-2 diabetic subjects

	Normal controls (mean ± SD)	Type-2 DM subjects (mean ± SD)	p-value
Plasma glucose (Fasting) mmol/L	3.9 ± 0.1	7.0 ± 2.7	0.0001
Plasma glucose (Post Prandial) mmol/L	5.7 ± 1.3	12.6 ± 4.6	0.0001
Glycated haemoglobin %	3.9 ± 0.7	6.8 ± 2.4	0.0001
Serum insluin (Fasting) pmol/L	132.2 ± 32.2	220.7 ± 252.3	0.04
Serum chromium (Fasting) nmol/L	44.7 ± 7.5	30.6 ± 9.3	0.0001
Serum total cholesterol (F) mmol/L	5.2 ± 1.1	5.4 ± 1.2	NS
Serum HDL cholesterol mmol/L	1.3 ± 0.2	1.4 ± 0.4	NS
Serum LDL cholesterol mmol/L	3.1 ± 1.5	3.5 ± 1.1	NS
Serum triglycerides mmol/L	1.1 ± 0.3	1.4 ± 0.9	.05

NS: Not significant

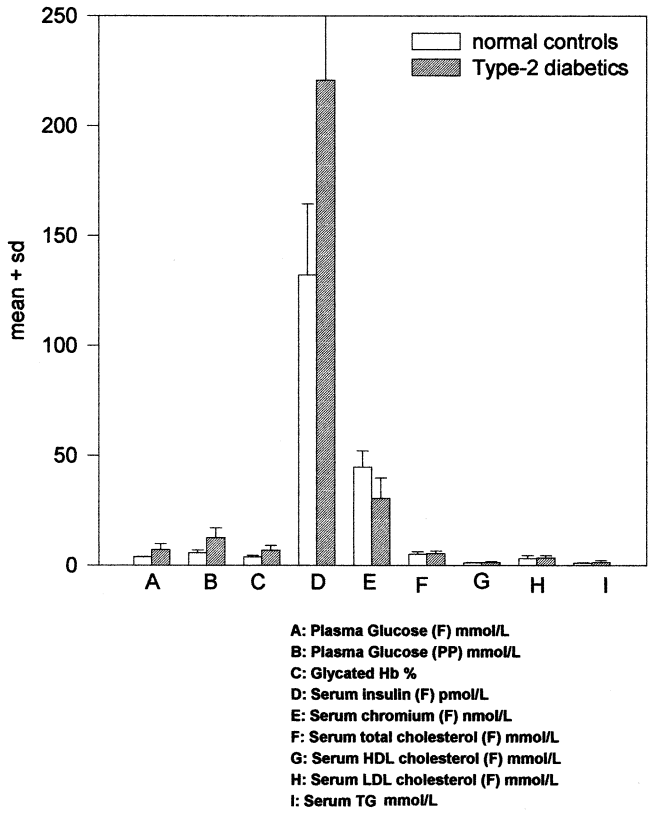

Fig. 1. Comparison of biochemical parameters between normal controls and type-2 diabetic subjects.

3. Results

This study, conducted between June 98 and April 99, included 50 patients at the outset. 50 normal healthy individuals were considered for comparison of physical and biochemical parameters. Out of the initial 50 patients, 43 completed the study. 4 patients had to be put on insulin regime due to uncontrolled hyperglycaemia and other complications, while the other three did not wish to continue on personal grounds.

Pre-treatment clinical parameters of normal controls and subjects showed that the Body Mass Index (BMI), Waist Hip Ratio (WHR) and systolic and diastolic blood pressures were higher in diabetics compared to controls (Table 1). Regarding the biochemical parameters, both fasting and post-prandial blood glucose values were expectedly higher among the subjects than in normal controls (Table 2). The mean fasting insulin value in the subjects was higher than the normal control level ($p < 0.05$) Significantly lower fasting chromium values were observed in the subjects in comparison to the normal controls (30.6 ± 9.4 nmol/L against 44.7 ± 7.5 nmol/L, $p < 0.0001$) (Figure 1). Considering the serum lipids, subjects had significantly higher TG values. Safety parameters (hemoglobin level, total and differential leukocyte counts, platelet count, serum urea, creatinine, ALT and alkaline phosphatase activity) showed no significant change after chromium administration.

Table 3 shows the different variables before and after

Table 3
Comparison of some relevant parameters (mean ± SD) in both placebo and chromium phases, before and after treatment with normal values

	Chromium phase		Placebo phase		Normal	P-value
	Before	After	Before	After		
Plasma glucose (Fasting) mmol/L	6.9 ± 2.5	6.4 ± 2.7	6.8 ± 2.7	7.2 ± 3.1	3.9 ± 0.1	0.001
Plasma glucose (Post Prandial) mmol/L	12.2 ± 3.6	10.2 ± 3.6	11.8 ± 5.1	11.8 ± 4.5	5.7 ± 1.3	0.001
Glycated haemoglobin	7.2 ± 2.5	7.2 ± 1.6	7.2 ± 1.9	7.9 ± 2.0	3.9 ± 0.7	0.04
Serum insulin (Fasting) pmol/L	256.3 ± 247.9	206.3 ± 179.2	159.3 ± 96.5	227.8 ± 217.4	132.2 ± 32.2	0.05
Serum chromium (Fasting) nmol/L	32.3 ± 7.5	35.7 ± 7.1	31.1 ± 7.1	31.1 ± 7.3	44.7 ± 7.5	0.0001
Serum total cholesterol (F) mmol/L	5.2 ± 1.9	4.5 ± 1.4	4.9 ± 1.6	4.6 ± 1.6	5.2 ± 1.1	0.69
Serum HDL cholesterol mmol/L	1.3 ± 0.3	1.1 ± 0.3	1.3 ± 0.4	1.1 ± 0.3	1.3 ± 0.2	0.99
Serum LDL cholesterol mmol/L	3.3 ± 1.7	2.8 ± 1.3	2.9 ± 1.3	2.8 ± 1.4	3.1 ± 1.5	0.71
Serum triglycerides mmol/L	1.5 ± 0.9	1.7 ± 0.9	1.5 ± 0.9	1.8 ± 0.9	1.1 ± 0.3	0.53

P-value: Differences in the percentage changes on mean value of initial reading after treatment between chromium and placebo phases.

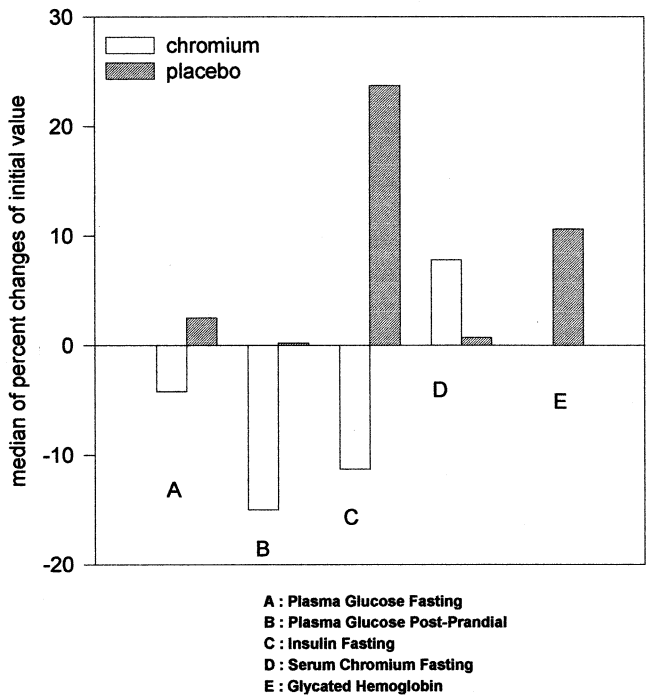

Fig. 2. Biochemical parameters showing significant change between placebo and chromium phases.

supplementation in the chromium and placebo phases and the p values refer to comparison of the mean change in the chromium and placebo groups. The following observations were made: BMI and WHR changes (not shown) were insignificant. Curiously, both systolic and diastolic blood pressures showed a reduction in both arms of the study, thus negating a significant influence of chromium on blood pressure (data not shown) The increase in the fasting serum chromium value following chromium supplementation (with no change observed in the placebo period) has acquired significance (p value = 0.001) Decrease of mean fasting and post-prandial serum glucose values by 0.44 mmol/L and 1.97 mmol/L in the chromium-phase against increase of + 0.45 mmol/L and 0.01 mmol/L in the placebo-phase period proved significant ($p < 0.001$ for both) The decreases in glycated hemoglobin and serum fasting insulin values after chromium supplementation against increases during the placebo-phase have rendered these changes significant ($p = <0.05$) (Figure 2). No significant differences were seen in changes in the lipid parameters between the chromium and placebo phases.

4. Discussion

The mammalian need for dietary Chromium, for maintenance of normal glucose tolerance, which was first postulated in 1957 [62] followed by another study in 1959 [63]. This prompted further studies in a variety of laboratory animals including rats, mice and squirrel monkey [64,65,66] and by the 60's the role of Chromium in animals had been established. However, the importance of Chromium in glucose metabolism and insulin sensitivity in humans was first assessed in 1977 [13], though suggested earlier by Mertz in 1969 [67]. Two other similar studies and some studies on children and elderly people established the role of Chromium in carbohydrate and lipid metabolism [14,15,16,17]. Chromium was identified as the active component of an organically complexed molecule called GTF (Glucose Tolerance Factor), the composition and function of which is still being investigated.

There is some clinical evidence that Chromium supplementation may decrease serum Tc, LDLc and TG levels and increase serum HDLc levels from findings based on some less well-controlled trials [18,20,68,69] and some controlled trials [70,71], while no significant changes in lipid profile was seen in some other studies [18,21,25].

Documentation of Chromium deficiency is difficult and the very small amounts of dietary Chromium needed are found in a variety of food and also as a contaminant from cookware. In the different experiments, chromium ion is assumed to be the active factor and a chelator is required to improve its bioavailability. Picolinic acid, a metabolite of tryptophan has been postulated to be the most useful chelator for effective bioavailability [72].

In the present double-blind study, the dose of Chromium used was twice that prescribed by the National Research Council in 1980 as adequate. Higher doses of Chromium picolinate, tried in a previous study [73], have been found to be effective. Safety of the drug has been proved in studies with doses equal to 5000 times the prescribed safe dose [74]. Two isolated cases who developed renal failure and hepatic dysfunction had been simultaneously receiving a number of other drugs known to cause renal toxicity [75, 76]. The subjects in the current study were mostly between 40–60 years of age, which is typical for type 2 diabetes mellitus patients all over the world [77,78] and with no gender propensity [79,80]. BMI and WHR, as markers of overall and central obesity [81,82], did not show significant change following chromium supplementation in this study.

In some previous studies diabetic patients were found to be chromium deficient [83,84]. But these studies were conducted mostly in economically prosperous countries where the general population has a low serum Chromium level. This is probably due to refinement and preservation of food [85] with little possibility of contamination during preparation of food and also due to increased stress [26,29]. In our study, Chromium levels were higher, both in normal healthy controls and the diabetics, in comparison to western studies. This may be due to natural contamination locally; however, in view of positive effects of chromium supplementation in this study, any contamination is likely to be due to biologically inactive forms of chromium. But the standard normal range is itself very wide with the upper limit being about 20 times the lower normal serum value (40.3 and 2.3 nmol/lit respectively) [50]. However, type 2 diabetics in this study

showed significant lower Chromium levels, compared to controls. A single Indian study had documented even higher Chromium levels among workers who drank water contaminated with minerals [86]. A previous study had shown blood Chromium values reflect body stores [87], hence fasting serum Chromium levels have been used for assessing chromium status in this study. This, expectedly, showed significant increase following Chromium supplementation ($p < 0.0001$); however, even after supplementation of Chromium picolinate at 200 μg twice daily fasting serum Chromium levels in individuals with diabetes were still significantly less than that of healthy controls ($p < 0.001$) Apart from in blood, Cr concentration even in hair, urine and other tissues or body fluids have been reported not to be reflective of Cr status [88]. Plasma Cr does not reflect tissue levels as tissue Cr levels are ten times higher than in plasma; raised plasma levels can be associated with a negative balance and hyperglycaemia may be associated with raised plasma Cr levels and increased urinary Cr excretion. Further, parenteral Cr supplementation in a patient receiving TPN was seen to culminate in exhausted tissue stores [89].

Effects of Chromium supplementation on glycaemic control have been evaluated by serum glucose values, glycated hemoglobin and insulin levels. Studies by Glinsmann-Hertz in 1966 and Nath et al. in 1979 [20,90] have reported beneficial effects of supplemental Chromium on glucose and its related parameters. Better results were seen, by double-blind study design, by Mossop [91], Kimura [92] and Thomas and Gropper [93].

In our study, there was significant improvement in glycaemic control in the Chromium-phase, in comparison to the placebo-phase. Higher pre-supplementation insulin level was seen in most of our subjects, as expected, and the post-supplementation decline, though modest, was statistically significant in comparison to controls ($p < 0.05$) The improved glycaemic control with decline in fasting serum insulin level suggests improved insulin sensitivity as the underlying mechanism. The improvement in insulin sensitivity was noted without any significant change in BMI or WHR. It may be noted that we were dealing primarily with non-obese type 2 diabetic subjects (only 14% had BMI >25); however, approximately half of them (49%) had WHR >0.9. It will be interesting to carry out similar studies in predominantly obese Indians with type 2 diabetes mellitus. Applicability of the results of this study to Western populations of type 2 diabetes mellitus is speculative.

Chromium supplementation has also been found to have a negative correlation with cardio-vascular diseases [88,94]. Many previous studies have found Chromium to favorably affect the lipid profile [70,71,93,95] while equivocal findings have been reported in some other [21,25,96]. In our study, changes in lipid parameters (total cholesterol, HDLc, LDLc and TG) did not show significant differences between the Chromium and placebo phases. A favorable effect may still be seen with Chromium supplementation at higher dosages and for longer period of follow-up [73]. No significant hepatic, renal or hematological changes were noted in our study, which is consistent with previous similar studies [74,97]. Though several studies with Cr picolinate have produced ambiguous results in respect to body mass, fat content, tissue size and blood variables [98], in view of some encouraging results from the current study, larger studies with longer follow-up are in order. Further, recent studies with LMWCr and Compound I, call for identification of the newer complexes and comparative studies of these with Cr picolinate.

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