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## METABOLIC PROCESSES

# Insulin sensitising action of chromium picolinate in various experimental models of diabetes mellitus

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## Abstract

Although chromium is an essential element for carbohydrate and lipid metabolism, its effects in diabetic patients are still debated. We have studied the effect of 6 week treatment with chromium picolinate (8 µg/ml in drinking water) in streptozotocin (STZ)-induced type 1 and type 2 diabetic rat models. The mechanism of anti-diabetic action of chromium picolinate was studied using C2C12 myoblasts and 3T3-L1 adipocytes. Chromium picolinate significantly decreased the area under the curve over 120 min for glucose of both STZ-induced type 1 (40 mg/kg, i.v. in adult rats) and type 2 (90 mg/kg, i.p. in 2 day old rat neonates) diabetic rats without any significant change in area under the curve over 120 min for insulin as compared to controls. The composite insulin sensitivity index and insulin sensitivity index ( $K_{ITT}$ ) values of both type 1 and type 2 diabetic rats were increased significantly by chromium picolinate. Treatment with chromium picolinate produced a significant decrease in elevated cholesterol and triglyceride levels in both types of diabetic rats. In 3T3-L1 adipocytes, chromium picolinate (0–10 µmol) per se did not produce any effect, however, when co-incubated with insulin it significantly increased the intracellular triglyceride synthesis ( $EC_{50} = 363.7$  nmol/l). Similarly in C2C12 myoblasts, chromium picolinate alone did not produce any effect, however, it significantly increased insulin-induced transport of <sup>14</sup>C-glucose. In conclusion, chromium picolinate significantly improves deranged carbohydrate and lipid metabolism of experimental chemically induced diabetes in rats. The mechanism of in vivo anti-diabetic action appears to be peripheral (skeletal muscle and adipose tissue) insulin enhancing action of chromium.

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**Keywords:** Chromium picolinate; Diabetes mellitus; Insulin resistance; 3T3-L1 adipocytes; C2C12 myoblasts

## Introduction

Insulin resistance has emerged as an important pathophysiological abnormality in diabetes mellitus.

Presence of insulin resistance in both type 1 and type 2 diabetes mellitus has been observed both clinically [1–3] as well as experimentally [4–6]. Absolute (as in case of type 1 diabetes mellitus) or relative (as in case of type 2 diabetes mellitus) lack of insulin is thought to render the target organs resistant to actions of insulin. Resistance to insulin action in skeletal muscle, adipose tissue and liver contributes to hyperglycemia as well as plays an

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important role in the pathogenesis of long-term complications [7]. Therefore, treatments that improve peripheral insulin resistance would be beneficial in the long-term management of these patients.

Chromium, a group VIb transition element, is an essential element required for normal carbohydrate and lipid metabolism [8]. Deficiency of chromium has been found to cause glucose intolerance which is an indicator of insulin resistance in humans [9,10] as well as experimental animals [11,12]. Studies in animals reported that chromium supplementation resulted in at least partial reversal of glucose tolerance [13–15]. However, when these studies were done in man, variable results were obtained. While various well-controlled clinical trials showed improvement in glucose tolerance [16–18], others found improvement only in 40–60% of the patients evaluated [19–21]. In some well-controlled clinical trials chromium failed to produce any response [22–25] but one of the studies rather reported deterioration of glucose control [26]. It has also been observed that subjects with good glucose tolerance who do not need additional chromium do not respond to supplemental chromium [9,27]. Subjects consuming adequate chromium and well balanced diets also do not respond to additional chromium [22]. Moreover, most of the investigators reporting beneficial effects with chromium supplementation in clinical [17,28,29] as well as experimental [30–32] set-up found that observed effects of chromium depended upon the existence of a deficiency state. Hence, it is claimed that chromium is a nutrient and not a drug and will therefore benefit only those who are deficient or marginally deficient in chromium [10]. Thus, although the essentiality of chromium in normal glucose homeostasis has been established, its usefulness as a therapeutic agent for the treatment of diabetes mellitus of variable etiology (other than chromium deficiency) remains questionable. Hence, the objective of the present investigation was to study the effect of chromium picolinate on insulin resistance in experimental models of diabetes mellitus.

## Material and methods

### Animals

Wistar rats from an inbred colony were bred under well-controlled conditions of temperature ( $22 \pm 2^\circ\text{C}$ ), humidity ( $55 \pm 5\%$ ) and a 12/12 h light-dark cycle with 07h30–19h30 being light phase. Conventional laboratory diet and tap water were provided ad libitum. The protocol of the experiment was approved by the Institutional Animal Ethical Committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA),

Ministry of Social Justice and Empowerment, Government of India.

### Induction of diabetes

Adult male Wistar rats weighing between 200–250 g were randomly divided into four groups : (i) control, (ii) control treated with chromium picolinate, (iii) type 1 diabetic control, (iv) diabetic treated with chromium picolinate. Diabetes was induced by single intravenous injection of streptozotocin (STZ) (Sigma Chemicals, USA), 40 mg/kg in 0.9% sodium chloride solution, via the tail vein under light ether anesthesia. The control group received an equivalent volume of 0.9% sodium chloride solution alone by the same route. 48 h after the injection of STZ, animals were checked for glucosuria using Diastix (Bayer Diagnostics, India) and those showing overt glucosuria ( $>2\%$ ) were considered as insulin dependent diabetic (IDDM) or type 1 diabetic.

Two-day-old male Wistar neonates were injected intraperitoneally with 90 mg/kg STZ in 0.9% sodium chloride solution. Control neonates received equivalent volume of 0.9% sodium chloride solution alone. The neonates were left with their own mothers and weaned at 4 weeks of age. Twelve weeks after the injection of STZ, animals were checked for fasting glucose levels. The animals showing fasting glucose levels  $>140$  mg/dl were considered as non-insulin-dependent diabetic (NIDDM) or type 2 diabetic. The experimental animals were divided into four groups as (i) control, (ii) control treated with chromium picolinate, (iii) type 2 diabetic control, (v) diabetic treated with chromium picolinate.

### Treatment protocol

Chromium picolinate was dissolved in water and administered at a concentration of  $8 \mu\text{g/ml}$  in the drinking water ad libitum for 6 weeks. The average chromium intake of the groups receiving chromium treatment was calculated according to the following formula:

$$\text{Chromium intake } (\mu\text{mol}) = \frac{\text{Concentration } (\mu\text{mol/ml}) \times \text{Fluid intake (ml)}}{\text{Rat weight (g)}}$$

The average chromium intake in type 1 diabetic rats was found to be  $11.19 \pm 0.01 \mu\text{mol/day}$ , in the case of type 2 diabetic rats  $3.57 \pm 0.07 \mu\text{mol/day}$  and in non-diabetic rats  $4.41 \pm 0.16 \mu\text{mol/day}$ .

Body weight and food intake were monitored regularly. Fluid intake was measured daily. Blood samples were collected after 6 weeks of drug treatment by nicking the tip of tail after 8 h fast. Serum was separated and analysed for glucose, cholesterol and triglycerides using diagnostic reagent kits (Bayer

Diagnosics, India). Serum insulin was estimated by radioimmunoassay technique using kits obtained from the Board of Radiation and Isotope Technology, Mumbai, India.

### Oral glucose tolerance test (OGTT)

At the end of 6 weeks of treatment, rats were subjected to oral glucose tolerance test (OGTT). Glucose (1.5 g/kg) was administered to 8 h fasted rats. Blood samples were collected from the tail vein at 0, 15, 30, 60 and 120 min after oral glucose administration. Serum was separated and analysed for glucose and insulin as explained earlier. Results of OGTT are expressed as integrated areas under the curves over 120 min for glucose (AUC<sub>g</sub>) and insulin (AUC<sub>i</sub>) calculated by using the trapezoid rule. Estimation of insulin sensitivity made from OGTT data was performed using the composite insulin sensitivity index (CISI) proposed by Matsuda and DeFronzo [33]. Calculation of the index was made according to the following equation:

$$\text{CISI} = \frac{10,000}{\sqrt{(\text{FSG} \cdot \text{FSI})(\text{MG} \cdot \text{MI})}}$$

where FSG and FSI are fasting serum glucose and insulin concentrations, respectively, and MG and MI are the mean glucose and insulin concentrations respectively over the course of OGTT.

### Insulin tolerance test (ITT)

At the end of 6 weeks, insulin sensitivity was measured by the insulin tolerance test described by Alford et al. [1]. 0.2 U/100 g body weight of purified porcine insulin (Actrapid, Novo Nordisk Pharma India Ltd.) was injected intravenously in 8 h fasted animals. Blood samples were collected from the tail vein at 0, 5, 10, 20 and 30 min after insulin administration. Serum was separated and analysed for glucose. The  $K_{\text{ITT}}$  as an index of insulin-mediated glucose metabolism, was calculated using the formula given by Lundbeak [34]:

$$K_{\text{ITT}} = \frac{0.693 \times 100}{t_{1/2}}$$

where  $t_{1/2}$  represents the half-life of plasma glucose decay, obtained by plotting plasma glucose concentration versus time on semi-logarithmic graph paper.

### Measurement of adipocyte differentiation

The 3T3-L1 fibroblasts (American Type Culture Collection, USA) were maintained in RPMI 1640 containing 5% fetal calf serum (FCS) (Gibco BRL, USA), 100 U/ml penicillin (Gibco BRL) and 100 µg/ml streptomycin (Gibco BRL), 0.5 µg/ml fungizone (Gibco

BRL) in a 75 cm<sup>2</sup> flask (Costar, USA) at 37°C in a humidified 95% air, 5% CO<sub>2</sub> atmosphere. Preadipocytes (1 × 10<sup>5</sup> cells per well) were cultured to confluency in six well plates for 2 days, then adipocyte differentiation was induced by the method of Shibata et al. [35]. Briefly, differentiation was initiated by treating confluent preadipocytes with 1 µmol dexamethasone and 0.5 mmol isobutylmethylxanthine (IBMX) (Sigma Chemicals, USA). After 2 days, the cells were given fresh medium containing chromium picolinate (0–10 µmol) in the presence and absence of 1 µg/ml insulin (Sigma Ltd., USA) and allowed to differentiate for additional 4 days. At the end of experiment, cells in the plate were harvested using rubber policeman. Scraped cells were transferred to 1.5 ml microcentrifuge tubes. The cell suspension was disrupted by sonication at maximum output for 15 s with a microtip. The intracellular triglycerides were determined using a Ponte Scientific colorimetric estimation kit, USA. Cell layer protein content was estimated according to the method of Lowry et al. [36]. Triglycerides were expressed as µg/mg protein.

### Glucose transport in C2C12 muscle cells

C2C12 murine myoblasts (American Type Culture Collection, USA) were cultured in 12 well plates in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL) supplemented with 10% fetal bovine serum (FBS) (Gibco BRL) containing 1 µg/ml gentamycin (Gibco BRL) at 37°C in a humidified 95% air, 5% CO<sub>2</sub> atmosphere. The medium was changed every 72 h. A day before the experiment, when the cells were confluent, they were incubated in DMEM without FBS. The glucose uptake by these cells was studied by the method of Hajduch et al. [37] for chromium picolinate (10 µmol) in the presence and absence of insulin (6 pmol/ml) using 2-(1,2-H)deoxy-D-glucose (2 µCi/ml) for 30 min. At the end of 30 min incubation, the cells were washed thrice with ice cold saline and further incubated for 45 min. The cells were solubilised in 200 µl of 0.5 mol/l sodium hydroxide for 40 min. The solubilised extracts were then processed for liquid scintillation counting.

### Statistical analysis

Data are presented as mean ± s.e. mean. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test. Data were considered statistically significant at  $P < 0.05$ .

## Results

### General characteristics

The general characteristics of the experimental animals at the end of 6 weeks of treatment schedule are summarised in Tables 1 and 2. Type 1 diabetic control animals exhibited significantly lower body weights as compared to their non-diabetic counterparts (Table 1). However, body weights of type 2 diabetic control rats were not significantly different from that of non-diabetic control rats (Table 2). Chronic treatment with chromium picolinate did not produce any significant effect on body weights of non-diabetic as well as type 1 diabetic and type 2 diabetic rats (Tables 1 and 2).

Symptoms of polyphagia and polydipsia, as evident from significantly greater food and fluid intakes of diabetic rats as compared to non-diabetic rats were observed in type 1 diabetic control animals (Table 1), whereas the food and fluid intake of type 2 diabetic rats was not significantly different from that of non-diabetic control rats (Table 2). Chronic treatment with chromium picolinate did not produce any significant effect on the body weight, food and fluid intake of either non-diabetic or type 1 and type 2 diabetic rats (Tables 1 and 2).

### Serum glucose and insulin levels and glucose tolerance

Fasting serum glucose levels in type 1 and type 2 diabetic rats were significantly higher than in corre-

sponding control rats (Tables 1 and 2, Figs. 1 and 2). After glucose load, the serum glucose levels over a period of 120 min in both type 1 and type 2 diabetic control groups were significantly higher than those in the corresponding non-diabetic control groups (Figs. 1 and 2). The AUCg was also significantly greater in type 1 and type 2 diabetic control groups as compared to their corresponding non-diabetic control groups (Tables 1 and 2). Fasting serum glucose levels of type 2 diabetic rats treated with chromium picolinate were significantly lower than type 2 diabetic control rats (Table 2, Fig. 2). However, no significant difference was observed in the fasting serum glucose levels of type 1 diabetic rats treated with chromium picolinate as compared to type 1 diabetic control rats (Table 1, Fig. 1). In non-diabetic control treated rats, fasting serum glucose levels as well as response to oral glucose load over a period of 120 min were significantly lower than non-diabetic control rats (Figs. 1 and 2). Mean AUCg of control-treated groups was lower than non-diabetic control groups, however, the difference was not statistically significant (Tables 1 and 2). When subjected to oral glucose challenge, significantly lower serum glucose levels were observed in chromium picolinate treated type 1 and type 2 diabetic groups than in respective diabetic control groups over a period of 120 min (Figs. 1 and 2). Chronic chromium treatment in both diabetic groups also produced significant reduction in AUCg as compared to their respective diabetic controls (Tables 1 and 2).

Fasting serum insulin levels and insulin response of type 2 diabetic control rats over 120 min after oral glucose load were not significantly different from that of the non-diabetic control group; however, serum insulin

**Table 1.** Effect of chronic treatment with chromium picolinate on general characteristics of the experimental animals resembling type 1 (IDDM) diabetes

Parameters	Groups			
	Control ( <i>n</i> = 8)	Control treated with chromium picolinate ( <i>n</i> = 8)	Type 1 control ( <i>n</i> = 7)	Diabetic treated with chromium picolinate ( <i>n</i> = 8)
Body weight (g)	250 ± 18	245 ± 8	166 ± 8*	169 ± 14
Food intake (g/day)	18 ± 4	16 ± 4	39 ± 5*	34 ± 6
Water intake (ml/day)	54 ± 5	61 ± 9	98 ± 10*	98 ± 14
Serum glucose (mg/dl)	54.8 ± 2.9	33.1 ± 2.9	324.2 ± 28.5*	306.3 ± 10.2
Serum insulin (μU/ml)	56.6 ± 5.8	54.3 ± 7.0	25.2 ± 5.5*	24.8 ± 2.2
Serum cholesterol (mg/dl)	101.5 ± 4.1	83.9 ± 4.1	130.7 ± 8.5*	96.2 ± 7.9 <sup>#</sup>
Serum triglyceride (mg/dl)	139.0 ± 8.4	117.0 ± 3.7	280.8 ± 25.4*	134.3 ± 3.8 <sup>#</sup>
AUCg (mg/dl 120 min)	9397 ± 876	6431 ± 556	75357 ± 3305*	56321 ± 1661 <sup>#</sup>
AUCi (μU/ml 120 min)	8245 ± 576	7196 ± 808	2581 ± 483*	3966 ± 781
CISI	2.47 ± 0.43	3.32 ± 0.28	0.95 ± 0.09*	1.24 ± 0.09
K <sub>ITT</sub>	6.38 ± 0.85	10.32 ± 1.42*	<1.16 ± 0.0*	1.90 ± 0.10 <sup>#</sup>

Each value is the mean ± s.e. mean; number of determinations in parentheses.

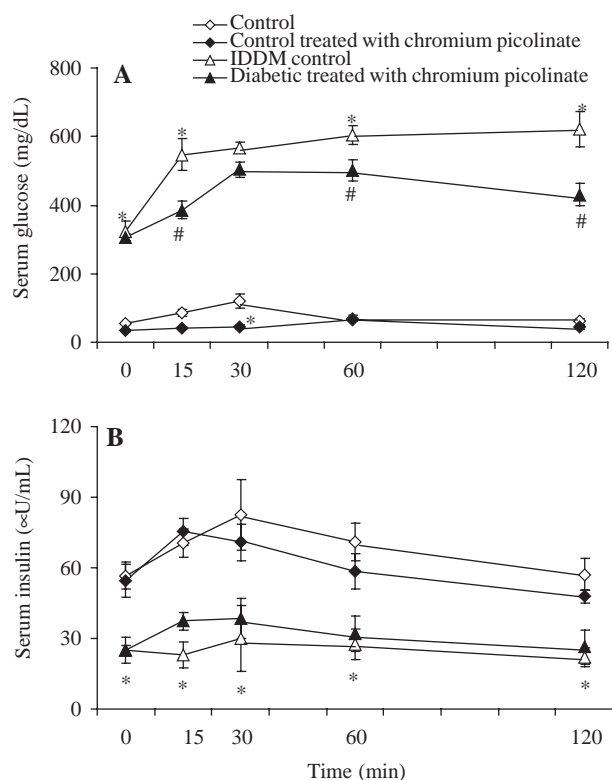
\*Significantly different from control; <sup>#</sup>significantly different from type 1 control; *P* < 0.05.

**Table 2.** Effect of chronic treatment with chromium picolinate on general features of the experimental animals resembling type 2 (NIDDM) diabetes

Parameters	Groups			
	Control ( <i>n</i> = 8)	Control treated with chromium picolinate ( <i>n</i> = 7)	Type 2 control ( <i>n</i> = 7)	Diabetic treated with chromium picolinate ( <i>n</i> = 8)
Body weight (g)	248 ± 19	246 ± 12	273 ± 20	279 ± 10
Food intake (g/day)	20 ± 5	16 ± 5	15 ± 4	14 ± 5
Water intake (ml/day)	54 ± 7	59 ± 9	50 ± 5	53 ± 4
Serum glucose (mg/dl)	56.4 ± 3.5	34.1 ± 3.0*	153.6 ± 5.0*	40.3 ± 5.0 <sup>#</sup>
Serum insulin (μU/ml)	54.0 ± 6.2	54.8 ± 8.2	51.8 ± 2.9	53.0 ± 2.7
Serum cholesterol (mg/dl)	101.5 ± 8.1	80.2 ± 4.6	147.1 ± 5.8*	86.4 ± 5.1 <sup>#</sup>
Serum triglyceride (mg/dl)	136.0 ± 6.4	110.8 ± 6.2	136.6 ± 6.9	101.7 ± 3.3 <sup>#</sup>
AUC <sub>g</sub> (mg/dl 120 min)	9048 ± 860	6390 ± 540	35400 ± 1440*	19150 ± 1734 <sup>#</sup>
AUC <sub>i</sub> (μU/ml 120 min)	8090 ± 560	7380 ± 810	7853 ± 211	8769 ± 545
CISI	2.50 ± 0.38	3.41 ± 0.033	0.88 ± 0.08*	2.37 ± 0.22**
K <sub>ITT</sub>	6.81 ± 0.75	9.82 ± 1.06*	3.45 ± 0.51*	7.76 ± 0.50 <sup>#</sup>

Each value is the mean ± s.e. mean; number of determinations in parentheses.

\*Significantly different from control; <sup>#</sup>significantly different from type 2 control; *P* < 0.05.



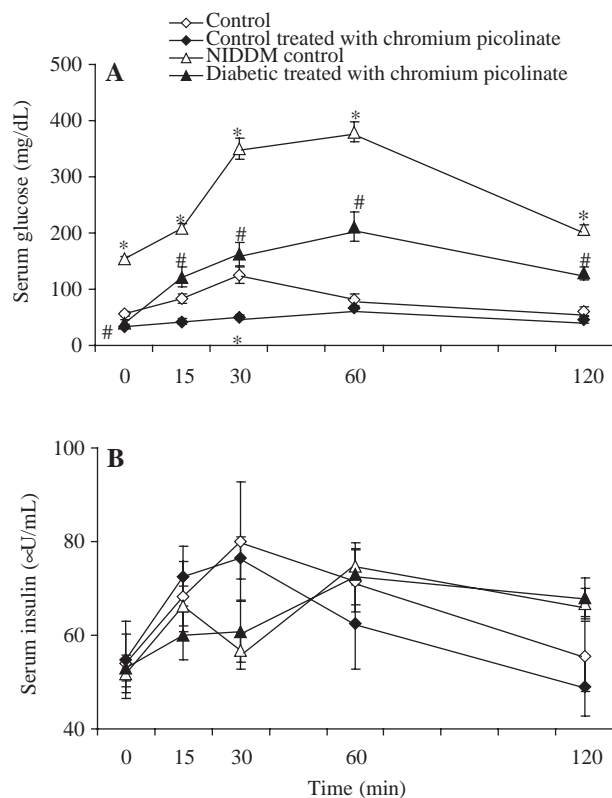
**Fig. 1.** Effect of chromium picolinate on serum glucose (A) and insulin (B) responses of type 1 (IDDM) diabetic rats to an oral glucose load (1.5 g/kg) during oral glucose tolerance test at the end of six weeks of treatment. Values are mean ± s.e. mean. \*Significantly different from control; <sup>#</sup>significantly different from diabetic control; *P* < 0.05.

levels and insulin response of the type 1 diabetic control rats were significantly lower as compared to the control group (Tables 1 and 2). The integrated insulin response (AUC<sub>i</sub>) of type 1 diabetic rats was significantly lower as compared to non-diabetic control rats (Table 1). The AUC<sub>i</sub> of type 2 diabetic control rats was not significantly different from that of non-diabetic control rats (Table 2). Chronic chromium treatment did not produce any significant effect on the basal insulin levels or insulin response to oral glucose load in non-diabetic control as well as diabetic treated groups as compared to the respective control groups. Treatment also did not produce any significant change in AUC<sub>i</sub> of non-diabetic as well as type 1 and type 2 diabetic rats (Tables 1 and 2).

### Insulin sensitivity

The composite insulin sensitivity index of both type 1 and type 2 diabetic rats was found to be significantly lower as compared to their respective control groups. Treatment with chromium picolinate significantly increased the CISI of type 2 diabetic rats (Table 2). Treatment also increased the CISI of control and type 1 diabetic rats; however, the difference was not statistically significant (Tables 1 and 2).

Insulin sensitivity measured as the rate of glucose disposal (K<sub>ITT</sub>) was significantly lower in type 1 and type 2 diabetic control animals as compared to their respective non-diabetic control groups (Tables 1 and 2). (Treatment with chromium picolinate significantly



**Fig. 2.** Effect of chromium picolinate on serum glucose (A) and insulin (B) responses of type 2 (NIDDM) diabetic rats to an oral glucose load (1.5 g/kg) during oral glucose tolerance test at the end of 6 weeks of treatment. Values are mean  $\pm$  s.e. mean. \*Significantly different from control; #significantly different from diabetic control;  $P < 0.05$ .

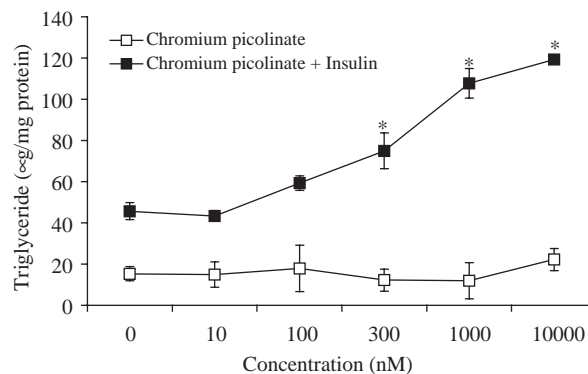
increased the  $K_{ITT}$  values of both non-diabetic control as well as diabetic rats as compared to their respective control groups (Tables 1 and 2).

### Serum cholesterol and triglyceride

Type 1 diabetic animals exhibited significantly higher cholesterol and triglyceride levels compared to non-diabetic control rats (Table 1). Type 2 diabetic rats showed significantly higher cholesterol levels as compared to non-diabetic control rats. However, there was no significant difference in the serum triglyceride levels of the two groups (Table 2). Chronic chromium picolinate treatment produced significant decrease in serum cholesterol and triglyceride levels of non-diabetic control as well as type 1 and type 2 diabetic rats as compared to corresponding non-diabetic control groups (Tables 1 and 2).

### 3T3-L1 cell differentiation

After 4 day-incubation of 3T3-L1 preadipocytes with chromium picolinate (0–10  $\mu$ mol) and insulin (1  $\mu$ g/ml),



**Fig. 3.** Effect of chromium picolinate on 3T3-L1 cell differentiation. The cells were cultured in medium containing fetal bovine serum (5%), dexamethasone (1  $\mu$ mol) and IBMX (0.5 mmol) for 2 days. The cells were then incubated with chromium picolinate (0–10  $\mu$ mol) in the presence and absence of insulin (1  $\mu$ g/ml) for 4 days. The cellular triglyceride levels were then measured. Data are the mean  $\pm$  s.e. mean of  $n = 3$ . \*Significantly different from no drug well.

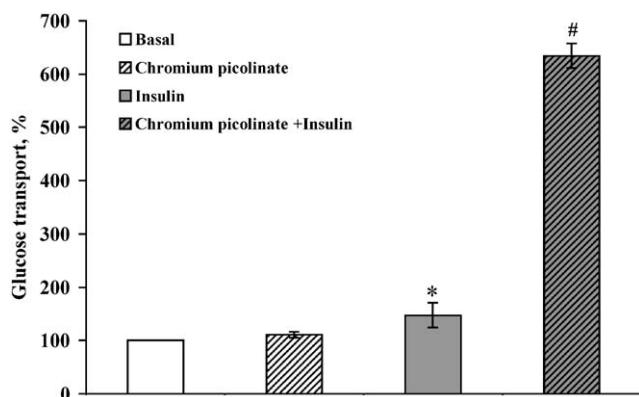
cellular triglyceride accumulation normalised by protein levels was measured as an index of the differentiated adipocyte phenotype. Incubation of cells with chromium picolinate alone failed to elevate triglyceride accumulation above that observed in the control cultures (Fig. 3). However, simultaneous incubation of cells with chromium picolinate and insulin resulted in dose-dependent increase in the accumulation of intracellular triglycerides though the increase was not significant at 10 and 100 nmol of chromium picolinate. However, a significant increase in the triglycerides was observed at 300 nmol and 1 and 10  $\mu$ mol of chromium picolinate with  $EC_{50}$  value of 363.7 nmol (Fig. 3).

### Glucose transport in C2C12 myoblasts

From the preliminary and the earlier reports by Hajduch et al. [37], it was found that the maximum uptake of glucose by insulin occurs at a concentration of 6 pmol/ml at 30 min. Chromium picolinate (10  $\mu$ mol) alone failed to produce any effect on basal glucose uptake in C2C12 myoblasts. However, co-incubation of chromium picolinate and insulin produced a significant increase in glucose uptake by the C2C12 cells over the basal (Fig. 4).

### Discussion

Chromium is reported to be an essential element required for normal carbohydrate and lipid metabolism [10]. Lack of dietary chromium has been reported to lead to the development of abnormal glucose tolerance



**Fig. 4.** Effect of chromium picolinate on glucose transport by muscle cells. C2C12 myoblasts were incubated with chromium picolinate and 2-(1,2-H)deoxy-D-glucose for 30 min and radiolabelled glucose in the cells was measured. Data are the mean  $\pm$  s.e. mean of  $n = 3$ . \*Significantly different from basal; #significantly different from insulin;  $P < 0.05$ .

in rats [1,4,13,38]. Moreover, it is reported that the impairment of glucose tolerance was the earliest recognised symptom of a low chromium state in animals [19]. However, in the present investigation corresponding non-diabetic control rats of both type 1 and type 2 diabetic rats exhibited normal glucose tolerance (Figs. 1 and 2). It has been reported that chromium deficiency is difficult to produce in animals [31] since it requires strict control of dietary (from food and water) as well as environmental sources of chromium (like steel cages and lids, nozzles of drinking water bottles, pipes supplying drinking water, etc.). Furthermore, it develops slowly over a period of several months [39]. In the present investigation, the intake of chromium by the experimental animals from these sources was not controlled. Although earlier studies have reported that individuals with diabetes mellitus were found to have lower chromium levels in serum [40], hair and tissues [41] as compared to non-diabetic subjects, it is claimed that chromium concentrations in hair, blood, urine and other body fluids and body tissues do not reflect chromium status [29]. In the present investigation, STZ induced type 1 and type 2 diabetic rats exhibited serum chromium levels that were not significantly different from their respective non-diabetic control rats (data not shown). Thus, all the observed metabolic derangements in diabetic (type 1 and type 2) rats in the present investigation could be attributed to STZ action rather than altered chromium status of these experimental animals. Various earlier studies have reported impairment in glycemic control due to chromium deficiency and its reversal with chromium supplementation in clinical [17,28,29] and experimental in vivo [31,32] as well as in vitro [30,42] settings. In contrast to these earlier reports, the observed effects of chromium

administration in the present investigation were found to be independent of the existence of chromium deficiency.

Chronic treatment with chromium picolinate significantly decreased glucose levels of non-diabetic and STZ-induced type 2 diabetic rats; however, it failed to do so in STZ-induced type 1 diabetic rats. Treatment with chromium picolinate significantly decreased the elevated AUCg in both type 1 and type 2 diabetic rats indicating improvement in glucose tolerance of treated animals. Decrease in AUCg in response to chromium picolinate treatment in type 2 diabetic rats was found to be 46%, whereas that in type 1 diabetic rats was found to be 25%. Improvement in glucose tolerance observed in the present investigation is consistent with earlier reports indicating that organically bound forms of chromium improved glucose tolerance in rats [14] and in diabetic humans [16,43]. However, these reports are unequivocal as some studies have reported no improvement even with organic chromium [23–25].

Decrease in the fasting glucose levels and AUCg of diabetic animals was not accompanied by any change in basal insulin levels or glucose stimulated insulin response during OGTT. However, some clinical studies have reported lower plasma insulin levels in response to chromium therapy [28,29,44]. Improvement in glucose tolerance without any change in basal insulin levels or insulin response during OGTT suggests that chromium may not have any effect on secretion of insulin from  $\beta$ -cells but possibly acts by enhancing insulin actions. This is further substantiated by the observation that chromium picolinate failed to lower elevated glucose level in type 1 diabetic rats which are insulinopenic unlike type 2 diabetic rats which have normal or higher insulin levels as compared to controls. Increase in insulin sensitivity was also found to be statistically significant in case of type 2 diabetic animals as indicated by CISI of Matsuda and DeFronzo [33]. This mathematical analysis was previously validated (with high correlation) against the hyperinsulinaemic-euglycemic clamp technique in 153 subjects with varying degrees of insulin resistance. Human trials have reported that chromium would be less effective or ineffective in those who were relatively insulinopenic or more glucose intolerant at the baseline [17]. Thus, insulinopenia in addition to severe glucose intolerance could explain failure of chromium picolinate to lower fasting glucose levels in type 1 diabetic rats despite improvement in glucose tolerance as observed during OGTT.

While OGTT provides an indirect measurement of insulin sensitivity, insulin tolerance test provides direct measurement of metabolic response to exogenous insulin. The insulin sensitivity index  $K_{ITT}$  measured during insulin tolerance test can assess the peripheral insulin resistance [1] which is a net result of resistance to insulin action at different sites including hepatic levels or

target tissue levels i.e. adipose tissue and skeletal muscles [7]. Chronic treatment with chromium picolinate increased the decreased glucose disposal in diabetic rats in response to exogenous insulin as evident from the significant increase in their  $K_{ITT}$  values. This effect was more pronounced in the case of type 2 diabetic rats as compared to that in type 1 diabetic rats. Treatment also increased the  $K_{ITT}$  values of non-diabetic rats. This further confirms the insulin sensitising action of chromium. These findings are consistent with those of Yoshimoto et al. [45] reporting enhancement in insulin responsiveness of STZ-induced diabetic rats while the insulin receptor number remained constant. Chromium was also reported to increase glucose utilisation and  $\beta$ -cell sensitivity measured during hyperglycemic clamp studies [46,47].

Above-mentioned results of *in vivo* studies clearly indicate insulin-sensitising action of chromium. From the current study, it appeared that the relative efficacy of chromium in the whole animal depends upon the presence of insulin. The differences in degree of severity of diabetic state and relative deficiency of residual circulating insulin could ultimately determine responsiveness to chromium treatment. A close dependence of the biological role of chromium on the presence of insulin has also been observed in many *in vitro* studies of chromium deficient tissues [14,30].

Existence of insulin resistance in both type 1 and type 2 diabetic rats can be observed in the present investigation. Impaired glucose tolerance in the face of normal basal insulin levels and normal insulin response to oral glucose load clearly indicate the insulin resistant state of type 2 diabetic animals in the present study. No glucose disposal even after 30 min of intravenous administration of exogenous insulin clearly indicates an insulin resistant state of these insulinopenic type 1 diabetic rats. Impaired uptake and utilisation of glucose by peripheral tissues (fat and muscle) represent major metabolic abnormalities of insulin resistance [7]. Since the skeletal muscle is the chief site of insulin mediated glucose disposal and conversion into fat is an important metabolic pathway for excess sugar, the observed effects of chromium in whole animals could be due to favourable effects in skeletal muscles. In the present investigation, insulin (6 pmol/ml) was found to significantly increase the glucose uptake in the skeletal muscle cell line C2C12 myoblasts. Chromium picolinate alone did not produce any effect on the basal glucose uptake; however, it significantly potentiated the insulin-stimulated glucose uptake in C2C12 myoblasts at a concentration of 10  $\mu$ mol indicating that chromium acts as an insulin enhancer. Thus, it is possible that chromium normalises the reduced peripheral glucose uptake by skeletal muscles and improves altered glucose homeostasis of diabetic animals.

The triglyceride content of the cells is used as the principal end point of preadipocyte differentiation in the 3T3-L1 cell differentiation assay. 3T3-L1 cells are a well established cell line that responds to physiological doses of insulin with increases in glucose uptake, glucose oxidation, glycogen synthesis and lipogenesis under *in vitro* conditions [48,49]. Chromium picolinate alone did not produce any significant effect; however, it significantly enhanced the cell differentiation in 3T3-L1 with  $EC_{50}$  value of 386.9 nmol in the presence of insulin suggesting that it is an insulin sensitiser. Chromium thus enhances the action of insulin on adipocytes by increasing intracellular triglyceride synthesis.

Deranged lipid metabolism in diabetes mellitus has been well established both clinically as well as experimentally. It has been reported that rats treated with STZ have increased plasma levels of triglycerides, cholesterol, free fatty acids and phospholipids [50]. In accordance with these reports, in the present investigation we observed elevated serum cholesterol and triglyceride levels in STZ-induced type 1 diabetic rats as well. STZ-induced type 1 diabetic rats showed significantly higher serum cholesterol levels compared to control rats; however, serum triglyceride levels of these animals were not significantly different from those of the controls.

Chronic chromium picolinate treatment significantly decreased serum cholesterol and triglyceride levels of both type 1 and type 2 diabetic as well as non-diabetic control rats indicating improvement in lipid metabolism. These findings are consistent with the earlier reports indicating decrease in hyperlipidaemia in genetically diabetic mice [15,41,51]. Abraham et al. [12] have reported reduction in the percentage of the aortic intimal surface covered by plaques in chromium treated rabbits. This improvement in lipid metabolism in the present investigation was independent of attainment of stable euglycemia in type 1 diabetic rats suggesting that fat metabolism was corrected even before carbohydrate metabolism. However, chromium supplementation in diabetic patients has been found to produce varied effects on lipid metabolism from no change in elevated lipid levels [21,22] to decrease in triglyceride and cholesterol with increase in HDL levels [52]. The decrease in total cholesterol, observed in the present investigation could be due to increased HDL cholesterol or decreased VLDL cholesterol. The triglyceride lowering effect of chromium is likely to be the result of enhancement of two separate actions of insulin: activation of lipoprotein lipase leading to an enhanced degradation of triglycerides and concurrent suppression of lipolysis [53] resulting in a decreased supply of free fatty acids required for triglyceride biosynthesis [54].

In the present investigation, chronic chromium therapy did not produce any significant change in the body weight as well as food and fluid intakes of both

type 1 and type 2 diabetic rats as compared to their respective diabetic controls indicating that the observed beneficial effects of chromium therapy in the present investigation were independent of the effects on body weight, food and fluid intake.

In conclusion, chromium picolinate, an organic form of chromium, was found to significantly improve deranged carbohydrate and lipid metabolism of experimental chemically induced diabetes in rats. The mechanism of in vivo anti-diabetic action appears to be a peripheral insulin enhancing action of chromium as evident from the potentiation of insulin-stimulated adipogenesis and glucose uptake at the insulin target organs in vitro. This study emphasises the therapeutic potential of chromium in the treatment of diabetes mellitus of etiology other than chromium deficiency. However, effects of chromium picolinate at various receptor and post-receptor levels of the insulin signaling cascade remain to be investigated.

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