# Chromium supplementation in impaired glucose tolerance of elderly: effects on blood glucose, plasma insulin, C-peptide and lipid levels

BY MATTI I. J. UUSITUPA[1], LEENA MYKKÄNEN[2], ONNI SIITONEN[2], MARKKU LAAKSO[2], HELENA SARLUND[2], PÄIVI KOLEHMAINEN[2], TIINA RÄSÄNEN[2], JORMA KUMPULAINEN[3] AND KALEVI PYÖRÄLÄ[2]

[1] *Department of Clinical Nutrition and* [2] *Department of Medicine, University of Kuopio, P.O. Box 1627, 70211 Kuopio, Finland,* [3] *Central Laboratory, Agricultural Research Centre of Finland, Jokioinen, Finland*

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**Altogether twenty-six elderly subjects (aged 65–74 years) with persistent impaired glucose tolerance (World Health Organization (1985) criteria) identified in a population-based study, were randomly treated either with chromium-rich yeast (160 μg Cr/d) or with placebo for 6 months. The 24 h urinary Cr increased from 0·13 (SE 0·03) to 0·40 (SE 0·06) μg/d in the Cr group (*n* 13) but no change was found in the placebo group (*n* 11) (0·13 (SE 0·02) *v.* 0·11 (SE 0·02) μg/d). No significant change was observed in the oral glucose tolerance test (glucose dose 75 g; 0, 1 and 2 h blood glucose respectively): 5·3 (SE 0·1), 9·3 (SE 0·3), 8·2 (SE 0·3) mmol/l *v.* 5·0 (SE 0·1), 8·5 (SE 0·4), 7·3 (SE 0·5) mmol/l in the Cr group; 4·9 (SE 0·2), 9·2 (SE 0·6), 8·1 (SE 0·3) mmol/l *v.* 4·8 (SE 0·2), 8·5 (SE 0·5), 7·0 (SE 0·6) mmol/l in the placebo group (baseline *v.* 6 months). Glycosylated haemoglobin, plasma insulin, C-peptide and apolipoprotein A1 and B levels remained unchanged, and no improvement was seen in serum total cholesterol (6·2 (SE 0·3) *v.* 6·4 (SE 0·3) mmol/l for the Cr group, 6·2 (SE 0·4) *v.* 6·5 (SE 0·3) mmol/l for the placebo group), high-density-lipoprotein-cholesterol (1·1 (SE 0·1) *v.* 1·2 (SE 0·1) mmol/l for the Cr group, 1·0 (SE 0·1) *v.* 1·1 (SE 0·1) mmol/l for the placebo group) or triacylglycerols (2·5 (SE 0·4) *v.* 2·0 (SE 0·4) mmol/l for the Cr group, 2·4 (SE 0·2) *v.* 2·5 (SE 0·2) mmol/l for the placebo group). The present results indicate that Cr supplementation does not improve glucose tolerance or serum lipid levels in elderly subjects with stable impaired glucose tolerance.**

**Chromium: Impaired glucose tolerance: Insulin: C-peptide: Serum peptide**

Trivalent chromium has been considered to be an essential trace element for normal glucose tolerance in rats (Schwarz & Mertz, 1959; Doisy *et al.* 1976; Mertz, 1979). Three patient reports have suggested that Cr deficiency may also lead to abnormal glucose tolerance and diabetes in patients with total parenteral nutrition deficient of Cr supplementation (Jeejeebhoy *et al.* 1977; Freund *et al.* 1979; Brown *et al.* 1986). Furthermore, there is some evidence coming from clinical trials that Cr could improve glucose tolerance and serum lipid profile in different patient groups or even in apparently healthy subjects (Glinsman & Hertz, 1966; Levine *et al.* 1968; Offenbacher & Pi-Sunyer 1980; Riales & Albrink, 1981; Anderson *et al.* 1983). Previous studies have, however, been uncontrolled (Offenbacher & Pi-Sunyer, 1988) or the effect of weight changes has not been taken into account (Offenbacher & Pi-Sunyer, 1980; Riales & Albrink, 1981). Despite the controversy concerning the significance of trivalent Cr in human nutrition various Cr-containing multivitamin – trace element pills are available. Since glucose tolerance becomes impaired with age (de Fronzo, 1981) and elderly people are considered to be at a particular risk of Cr

deficiency (Offenbacher & Pi-Sunyer, 1988), we carried out a placebo-controlled long-term study with the aim of examining the effect of Cr supplementation in elderly subjects who had a stable impaired glucose tolerance (IGT) on the basis of World Health Organization (1985) criteria.

## SUBJECTS AND METHODS

### *Subjects*

The subjects for the present study were recruited from a large epidemiological study in which the prevalence of IGT and diabetes as well as the occurrence of cardiovascular diseases were examined among a random sample of 1300 elderly people in the age-group of 65–74 years (Mykkänen *et al.* 1989). From the study of Mykkänen *et al.* (1989), sixty-seven subjects with IGT on the basis of World Health Organization (1985) criteria participated in a second oral glucose-tolerance test, and only those twenty-six subjects who showed a persistent abnormal glucose tolerance were eligible for the present study. They were randomly allocated to either Cr-supplementation group or placebo group. One subject in each group did not complete the trial. From those twenty-four subjects who completed the trial the Cr group comprised thirteen subjects (six men, seven women) and the placebo group eleven subjects (six men, five women). The mean age (years) of men in the Cr group was 71 (SE 1·0) and women 68 (SE 0·6). The respective values for men and women in the control group were 68 (SE 0·9) and 68 (SE 1·5) years (the difference between the groups was not significant). There were no significant differences in the mean body mass index between the two groups, and the 24 h urinary Cr excretion was similar in both groups before the trial (Table 1).

Five subjects in the Cr group and six in the placebo group were treated with a $\beta$-blocking agent. Seven subjects in the Cr group and two subjects in the placebo group were receiving diuretic therapy. The doses of these drugs were kept unchanged during the trial. Furthermore, the subjects were asked not to change their dietary habits while participating in the study, but there was no estimate of usual dietary Cr intake of the subjects.

### *Study design*

The study was carried out by a double-blind, parallel-group design. After the second oral glucose-tolerance test the eligible subjects were recruited for the study. The trial lasted for 6 months, and the following measurements were performed before the trial and then at 3 and 6 months: height (at the beginning), body-weight, blood pressure, 24 h urinary Cr excretion, 2 h oral glucose-tolerance test (glucose dose 75 g) with fasting, 1 and 2 h samples for blood glucose, plasma insulin and C-peptide determinations, glycosylated haemoglobin (HbA1c), serum and lipoprotein cholesterol levels, serum total triacylglycerol and apolipoproteins A1 and B.

Organic trivalent Cr was administered as four pellets of Cr-rich yeast daily. The supplementation dose of Cr was 160 $\mu$g/d. The control group received identical placebo pellets with the dose of four pellets daily.

### *Ethical considerations*

The study was approved by the Ethics committee, University of Kuopio. Each subject gave their informed consent for the study.

### *Methods*

Body-weight was measured in light clothes without shoes after an overnight fast, using a weighing machine (Seca; Dayton Vaaka Oy, Espoo, Finland). Blood pressure was measured in the sitting position from the right arm after a 5 min rest with a mercurosphygmomanometer (cuff size 125 × 400 mm).

Blood glucose was measured using a glucose oxidase (*EC* 1.1.3.4) method (Glucose Auto & Stat HGA-1120 analyzer; Daiici Co, Kyoto, Japan). Plasma samples for the determination of insulin and C-peptide were stored at −20° until required for analysis. Insulin was analysed by a radioimmunoassay (Phadeseph Insulin RIA 100; Pharmacia Diagnostics AB, Uppsala, Sweden). C-peptide was determined by a radioimmunoassay (C-peptide of insulin 125 J RIA kit; Incstar Co, Stillwater, MN, USA). Glycosylated HbA1c was measured with a commercial fast protein liquid chromatograph with reference values from 4 to 6% in non-diabetic subjects (Pharmacia Fine Chemicals AB, Uppsala, Sweden). Serum and lipoprotein lipids were determined from 12-h overnight fasting samples. Lipoprotein fractionation was performed by ultracentrifugation at a density of 1·006 to remove very-low-density lipoprotein (VLDL), followed by precipitation of LDL from the infranatant fraction by dextran sulphate and magnesium chloride (Penttilä *et al.* 1981). Enzymic methods were used for the determination of cholesterol (Röschlau *et al.* 1974) and triacylglycerols (Wahlefeld, 1974). Cholesterol was determined from the whole serum, the infranatant and from the supernatant fraction after precipitation of low-density lipoprotein (LDL). LDL-cholesterol was calculated as the difference between the mass of cholesterol in infranatant and in high-density lipoprotein (HDL); VLDL-cholesterol was also calculated. Apolipoproteins A1 and B were analysed by an immunoprecipitation method (Avogaro *et al.* 1979; Fruchart *et al.* 1982) using commercial reagent kits (Kone, Diagnostics, Helsinki).

The subjects received detailed oral and written instructions about how to collect 24 h urine samples. The samples were collected into acid-washed 2 l polyethylene bottles. At each visit the adequacy of sample collection was ascertained and the volume measured.

For the determination of Cr in 24 h urine samples a Perkin-Elmer 5000/Zeeman atomic absorption spectrometer equipped with a Zeeman HGA-500 graphite furnace, a model AS-40 autosampler, a model 3600 data station (Perkin-Elmer) and a Canon model AP-500 printer were employed. Samples were analysed directly by the method of additions (Kumpulainen *et al.* 1983). We participated in an interlaboratory comparison study organized by J. Versieck on the level of Cr in a human serum reference material (RM) and obtained a value of 0·70 (SE 0·11) ng Cr/g dry weight compared with a recently certified value of 0·76 (SE 0·11) ng Cr/g dry weight (Versieck *et al.* 1988). As a routine control sample we used a Seronorm TM Urine RM (batch no. 108) and obtained a mean value of 20 (SE 1) ng Cr/ml which is comparable with a preliminary recommended value of 22 ng/ml (Nycomed AS Diagnostics, PO Box 4284 Torshov, 0401 Oslo 4, Norway).

### *Statistical methods*

Data analyses were carried out at the Kuopio University Computer Center using the SPSSX programmes. Within-group comparisons of the changes in variables during the trial were carried out by multivariate analysis of variance (MANOVA). Between-group comparison of Cr excretion values was also carried out using MANOVA. Between-group comparisons were made using Student's two-tailed *t* test for independent samples (baseline data only). Otherwise multivariate analysis of covariance (MANOVA) was used in order to adjust the effect of the change in body mass index on glucose, insulin, C-peptide and lipid values. The values given in the results are unadjusted. Insulin was analysed after logarithmic transformation.

## RESULTS

### *Body mass index and blood pressure*

No significant change was found in the mean body mass index in the placebo group, but body mass index declined slightly in the Cr group from 29·9 kg/m$^2$ to 29·1 kg/m$^2$ ($P < 0·05$; Table 1). There was no difference between the groups in the mean blood pressure

Table 1. *Body mass index ($kg/m^2$), and 24 h chromium excretion ($\mu g$) of elderly Finnish subjects with or without Cr supplementation*

(Mean values with their standard errors)

| Treatment group† | Before trial Mean | Before trial SE | 3 months Mean | 3 months SE | 6 months Mean | 6 months SE | Statistical significance of difference‡: *P* |
|---|---|---|---|---|---|---|---|
| Body mass index | | | | | | | |
| Placebo | 30·3 | 0·8 | 30·0 | 0·8 | 29·9 | 1·0 | |
| Cr | 29·9 | 2·7 | 29·4 | 2·7 | 29·1 | 2·8 | < 0·05 |
| Cr excretion | | | | | | | |
| Placebo | 0·13 | 0·02 | 0·15 | 0·04 | 0·11 | 0·02 | |
| Cr | 0·13 | 0·03 | 0·48* | 0·14 | 0·40** | 0·06 | < 0·001 |

Between-group difference at 3 and 6 months was significantly different: * $P = 0·045$; ** $P < 0·001$.
† For details, see p. 210.
‡ Within-group change over the study period (by multivariate analysis of variance).

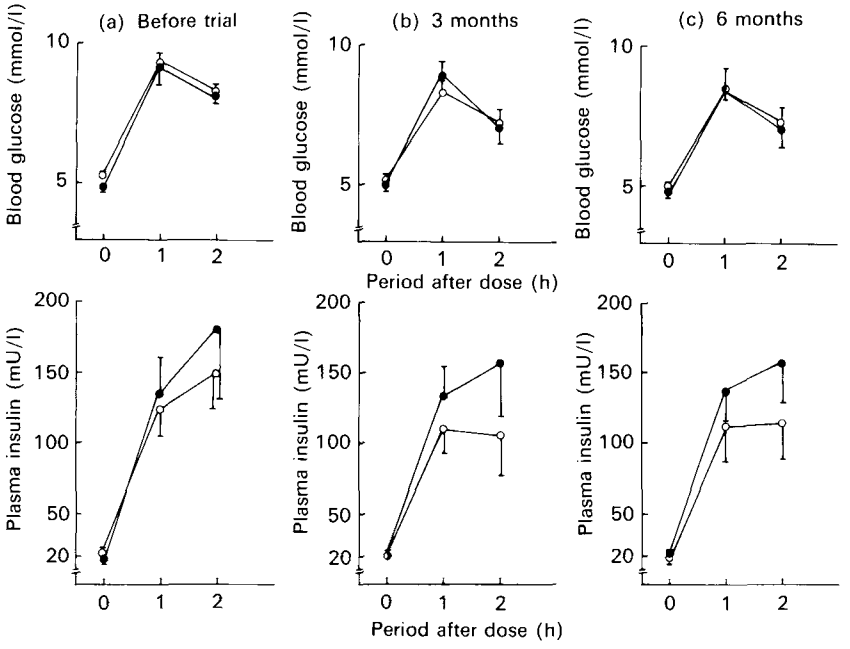


Fig. 1. Blood glucose and serum insulin of elderly Finnish subjects, with (○——○) or without (●——●) chromium supplementation, in oral glucose tolerance tests, before the trial and at 3 and 6 months. For details of subjects and procedures, see p. 210. Points are mean values with their standard errors represented by vertical bars.

level, and blood pressure levels remained unchanged in both groups during the trial (values not shown).

*Glucose tolerance and insulin secretion*

No significant changes were observed in the fasting or post-load blood glucose and insulin values during the trial in either group (Fig. 1), and there were no significant differences between the groups in these variables at the baseline or at 3 and 6 months. At 3 and 6

Table 2. *Glycosylated haemoglobin (HbA1c) (%), and C-peptide (nmol/l) in an oral glucose tolerance test in elderly Finnish subjects with or without chromium supplementation*

(Mean values with their standard errors)

| | Before trial | | | | 3 months | | | | 6 months | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment group*... | Placebo | | Cr | | Placebo | | Cr | | Placebo | | Cr | |
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Glycosylated HbA1c | 5·3 | 0·2 | 5·4 | 0·3 | 5·8 | 0·3 | 5·4 | 0·3 | 6·1 | 0·2 | 5·8 | 0·2 |
| C-peptide: | | | | | | | | | | | | |
| Fasting | 1·19 | 0·1 | 1·29 | 0·2 | 1·28 | 0·1 | 1·13 | 0·2 | 1·33 | 0·1 | 1·26 | 0·2 |
| 1 h post-glucose | 4·19 | 0·38 | 3·63 | 0·4 | 4·02 | 0·3 | 3·78 | 0·4 | 4·14 | 0·3 | 3·48 | 0·3 |
| 2 h post-glucose | 4·79 | 0·59 | 4·39 | 0·4 | 4·85 | 0·6 | 3·76 | 0·4 | 4·39 | 0·4 | 4·30 | 0·4 |

* For details, see p. 210.

months, post-glucose plasma insulin levels tended, however, to be lower in the Cr group than in the placebo group, but this was attributable to the weight loss which was statistically significant in the Cr group only (MANCOVA). In three subjects of the Cr group an initial high 2 h post-glucose insulin level declined markedly (from 370 to 211, from 169 to 66 and from 222 to 54 mU/l, baseline *v.* 6 months). A similar finding was recorded for one subject of the control group (612 *v.* 261 mU/l). Only one subject (in the Cr group) of these four subjects showed a weight loss of $> 2$ kg. Otherwise the changes in post-glucose insulin levels were related to body-weight changes.

Table 2 shows the results of glycosylated HbA1c and fasting and post-glucose C-peptide levels at baseline, 3 and 6 months. No significant changes were found in these variables during the study within or between the placebo and Cr groups.

### *Serum and lipoprotein lipids*

Serum and lipoprotein-cholesterol and serum total triacylglycerols and apolipoproteins A1 and B showed no statistically significant changes during the trial in either group, and no significant differences were found between the groups (Table 3). Serum triacylglycerol levels were, however, somewhat lower in the Cr group at the end of the study.

## DISCUSSION

Since the finding that trivalent Cr was an essential trace element for normal glucose tolerance in rats and that Cr deficiency could also be involved in lipid disturbances (Schwarz & Mertz, 1959; Doisy *et al.* 1976; Mertz, 1979; Offenbacher & Pi-Sunyer, 1988), numerous studies have been carried out to examine the effects of Cr supplementation on glucose tolerance and serum lipid values in different subject groups (Glinsman & Mertz, 1966; Levine *et al.* 1968; Offenbacher & Pi-Sunyer, 1980, 1988; Riales & Albrink, 1981; Anderson *et al.* 1983). The results of these previous studies have been variable but, in general, studies with adequate placebo periods or control groups have failed to show any benefit in terms of glucose tolerance or plasma lipids among diabetic subjects (Sherman *et al.* 1968; Rabinowitz *et al.* 1983; Uusitupa *et al.* 1983; Offenbacher *et al.* 1985).

The present study was designed to investigate whether a persistent IGT in elderly subjects, who are suggested to be in particular risk of Cr deficiency (Offenbacher & Pi-Sunyer, 1988), could be improved by long-term Cr supplementation. Furthermore, the

Table 3. *Serum lipids (mmol/l) and apolipoproteins (apo) A and B (g/l) in elderly Finnish subjects with or without chromium supplementation*

| Treatment group*... | Before trial | | | | 3 months | | | | 6 months | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Placebo | | Cr | | Placebo | | Cr | | Placebo | | Cr | |
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Total-C | 6·2 | 0·4 | 6·2 | 0·3 | 6·2 | 0·2 | 6·4 | 0·3 | 6·5 | 0·3 | 6·4 | 0·3 |
| VLDL-C | 1·1 | 0·1 | 1·1 | 0·2 | 1·0 | 0·1 | 1·2 | 0·2 | 1·0 | 0·1 | 1·0 | 0·2 |
| LDL-C | 4·1 | 0·3 | 4·0 | 0·2 | 4·1 | 0·2 | 4·2 | 0·2 | 4·5 | 0·3 | 4·3 | 0·3 |
| HDL-C | 1·01 | 0·09 | 1·07 | 0·09 | 1·07 | 0·06 | 1·16 | 0·08 | 1·10 | 0·08 | 1·17 | 0·09 |
| Triacylglycerols | 2·4 | 0·2 | 2·5 | 0·4 | 2·5 | 0·2 | 2·2 | 0·4 | 2·5 | 0·2 | 2·0 | 0·4 |
| Apo A | 1·3 | 0·1 | 1·3 | 0·05 | 1·3 | 0·04 | 1·4 | 0·05 | 1·4 | 0·04 | 1·4 | 0·04 |
| Apo B | 1·2 | 0·1 | 1·2 | 0·1 | 1·3 | 0·1 | 1·2 | 0·1 | 1·3 | 0·1 | 1·2 | 0·1 |

Total-C, total cholesterol; VLDL-C, very-low-density-lipoprotein-cholesterol; LDL-C, low-density-lipoprotein-cholesterol; HDL-C, high-density-lipoprotein-cholesterol.

* For details, p. 210.

effect of Cr supplementation on insulin secretion and serum lipids and apolipoproteins A1 and B were examined. The study also contained a placebo group and, to evaluate the intake of Cr, 24 h urinary Cr was regularly monitored in each subject. The 3–4-fold increase in urinary excretion in Cr-treated subjects indicated that they were in positive Cr balance, while in the placebo group urinary Cr excretion remained stable during the study.

In accordance with most other controlled studies (Sherman *et al.* 1968; Rabinowitz *et al.* 1983; Uusitupa *et al.* 1983; Offenbacher *et al.* 1985) we were unable to find any evidence for an improved glucose tolerance attributable to Cr supplementation. Furthermore, no significant change in glycosylated HbA1c values or insulin response to the glucose load was observed after Cr supplementation. The plasma C-peptide level which reflects more closely insulin secretion also remained unchanged. The decline in post-glucose plasma insulin values could be explained by a concomitant decrease in body-weight of the subjects receiving Cr and random variation in individual subjects. It can be argued that the supplementation period of the present study was not long enough, but it was longer than in most previous studies on this field.

The serum lipids and apolipoproteins A1 and B were not affected by Cr treatment. This is in accordance with our previous study on patients with non-insulin-dependent diabetes in whom no significant changes were seen after Cr supplementation (Uusitupa *et al.* 1983). Furthermore, the changes in serum lipids in other studies have been inconsistent (Offenbacher *et al.* 1980, 1988; Riales & Albrink, 1981) and at least in some studies they could be due to body-weight changes (Riales & Albrink, 1981).

In a previous study elderly subjects were reported to show some improvement in glucose tolerance after supplementation with Cr-rich brewer's yeast, while in the control group who received Cr-poor torula yeast no change in glucose tolerance was seen (Offenbacher & Pi-Sunyer, 1980). Unfortunately, possible body-weight changes in the patients were not reported which leaves this study open to criticism. In another study Cr supplementation resulted in better glucose tolerance and an increase in HDL-cholesterol levels in healthy men, but a concomitant decrease in body-weight could explain these findings (Riales & Albrink, 1981). Anderson *et al.* (1983) found improved glucose tolerance after Cr supplementation only in a group of subjects who initially had blood glucose values greater than 100 mg/dl at 90 min during an oral glucose tolerance test, but only one glucose

tolerance test was carried out before Cr supplementation and, furthermore, the result was based on secondary analysis of the data. It should be emphasized that there is a marked random variation in glucose tolerance; in the present study forty-one of sixty-seven subjects with IGT showed a normalization of glucose tolerance even without any intervention.

The studies suggesting that Cr is an essential trace element for normal glucose tolerance in animals were carried out before a reliable method for the determination of Cr concentration in biological samples was available (Anderson, 1981; Offenbacher & Pi-Sunyer, 1988). Furthermore, in one recent experimental study physiological Cr supplementation failed to show any improvement in glucose tolerance or plasma cholesterol in Cr-deficient rats (Donaldson *et al.* 1985). On the other hand, human milk is very low in Cr (0·3 $\mu$g/l; Kumpulainen *et al.* 1980) and recently apparently healthy Finnish children who died as the result of an accident have been found to have particularly low liver Cr concentrations (Vuori & Kumpulainen, 1987). These findings also cast some doubt on the essentiality of Cr. Furthermore, the biologically active form of Cr is still unidentified and only little is known about the physiological role of Cr. Cr has been said to be an essential part of the glucose tolerance factor facilitating the action of insulin (Schwarz & Mertz, 1959; Doisy *et al.* 1976; Mertz, 1979; Anderson, 1981; Offenbacher & Pi-Sunyer, 1988), but the existence of this factor remains to be determined.

Our results indicate that Cr supplementation in elderly subjects with stable impaired glucose tolerance and a particularly low daily Cr intake (Kumpulainen *et al.* 1980; Varo & Koivistoinen, 1980) does not result in the normalization of glucose and insulin metabolism, and has no benefit with respect to serum lipid levels. They also suggest that Cr deficiency does not seem to be an important risk factor for impaired glucose tolerance in elderly subjects in Finland where the mean daily intake of Cr remains substantially lower ($< 30$ $\mu$g) (Kumpulainen *et al.* 1980; Varo & Koivistoinen, 1980) than that of 50–200 $\mu$g recommended by the National Research Council (1989).

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