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Chromium-Induced Glucose Uptake, Superoxide Anion Production, and Phagocytosis in Cultured Pulmonary Alveolar Macrophages of Weanling Pigs

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ABSTRACT

The dose-dependent effects of chromium chloride ($CrCl_3$) and chromium picolinate (CrPic) were evaluated for their glucose uptake, superoxide anion (O_2^-) production, activity of glucose-6-phosphate dehydrogenase, and phagocytosis of incubated pulmonary alveolar macrophages in medium containing no or $5 \times 10^{-8}M$ insulin. Glucose uptake was found to increase in cells treated with 20 μg/L $CrCl_3$. Incubation with 20 μg/L of CrPic enhanced glucose uptake and O_2^- production in an insulin-dependent manner. However, the inclusion of CrPic to 100 μg/L in the medium absent of insulin also increased O_2^- production. The activity of glucose-6-phosphate dehydrogenase was not affected by either the addition of Cr or insulin. The phagocytosis of *Escherichia coli* by macrophages was enhanced significantly ($p < 0.05$) in medium containing 10–100 μg/L $CrCl_3$ or 20–100 μg/L CrPic in the presence of insulin. These results suggest that the addition of 10–20 μg/L $CrCl_3$ enhances directly the cellular activity of

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macrophages, whereas the effect of CrPic requires the cooperative action of insulin in enhancing their glucose uptake and phagocytosis.

Index Entries: Chromium; weanling pigs; macrophage; glucose uptake; superoxide anion; phagocytosis.

INTRODUCTION

The trace element chromium (Cr) was first proposed in 1959 by Schwarz and Mertz *(1)* to maintain normal glucose tolerance. Recently, Cr has been recognized as an essential dietary mineral required for normal metabolism of carbohydrate, proteins, and lipids in livestock and laboratory animals *(2,3)*. These beneficial actions of supplemental Cr have traditionally been attributed to a general improvement in insulin sensitivity and glucose tolerance in peripheral tissues *(2,4)*. Several investigations using growing pigs and cows have shown that glucose homeostasis was more stable with both glucose and insulin challenges in the chromium picolinate (CrPic)-supplemented group *(5,6)*.

Previous work from this *(7,8)* and other laboratories *(9)* has suggested that dietary CrPic supplementation inclines to increase the levels of immunoglobulins and anti-*Pseudorabies* virus titer and to alleviate stress caused by lipopolysaccharide challenge in weanling pigs. However, the mechanisms of Cr-enhanced immune responses have not been established.

The blastogenesis of peripheral blood mononuclear cells (PBMC) was found to be enhanced in dairy cows supplemented with dietary Cr *(10)*. The PBMC culture medium containing serum from Cr-fed cows also induced the enhanced blastogenesis, however; the hormonal profiles in serum revealed no change in this trial *(11)*. Furthermore, it has been reported that the addition of Cr from chelated Cr or $CrCl_3$ increased blastogenesis and that the level of chelated Cr needed to enhance blastogenesis was higher than that of $CrCl_3$ in the presence of insulin *(12,13)*. Evans and Bowman *(14)* reported that glucose and amino acid uptake by muscle cells was more efficient with CrPic than that with chromium nicotinate or $CrCl_3$ in the presence of insulin. Therefore, we postulated that the different forms of Cr inducing cellular activity of immune cells might be by way of different pathways.

Macrophages are the important immune cells acting on phagocytosis and respiratory burst *(15)*. The increased level of glucose in culture medium is related to the increased capacity of phagocytosis and the level of interleukin-1β (IL-1β) production *(16,17)*. The aim of the present study was to examine the relationship among various Cr compounds and insulin by investigating the influence of the dose-dependent effects of CrPic and $CrCl_3$ in the presence or absence of insulin on glucose uptake, superoxide anion (O_2^-) production, activity of glucose-6-phosphate dehydrogenase, and phagocytosis in cultured pulmonary alveolar macrophages of weanling pigs.

MATERIALS AND METHODS

Cell Culture

Macrophages used in the study were isolated from pulmonary alveoli of 6 to 8 wk-old crossbred weanling pigs as described previously by Hsiao *(18)*. The macrophages collected from the tissues were washed and resuspended in RPMI 1640 medium (Life Technologies, Inc., USA) containing 10% (v/v) dimethylsulfoxide (DMSO) (Sigma Chemical Co., USA) and 10% heat-inactivated fetal bovine serum (FBS) (Life Technologies, Inc.) before freezing at –140°C. When performing the assay, macrophages were thawed and resuspended in Dulbecco's modified Eagle medium (DMEM; Life Technologies, Inc.) with 10 m*M* glucose, 2% heat-inactivated FBS, and 1% antibiotic–antimycotic solution (Life Technologies, Inc.). Cells were grown in an incubator under 5% CO_2 atmosphere at 37°C for 12 h, and then washed and suspended in DMEM with 2 m*M* glucose. The macrophages were then incubated with chromium chloride ($CrCl_3$; Sigma Chemical Co.) or chromium picolinate (CrPic; Prince Co., USA) in 0–100 µg/L Cr medium containing no or 5×10^{-8}*M* insulin for 4 h at 37°C.

Glucose Uptake Studies

The glucose uptake of macrophages was measured according to the method of Morris et al. *(19)*. After incubating 1×10^6 cells/well ($n = 8$) in a 24-well microplate with Cr and insulin for 4 h, the medium was aspirated and cells were washed three times with DMEM. The uptake of glucose was initiated by the addition of 200 µL DMEM containing 0.2 µCi of 2-deoxy-D-[1-^{3}H] glucose (specific activity 16.0 µCi/mmol, Amersham Life Sciences, UK) with "cold" anhydrous glucose as the carrier (2 mmol/L). This produced a final volume per well of 2 mL and a final glucose concentration of 200 µmol/L. Glucose uptake was allowed to continue for 15 min at 37°C. After that, the cells were washed rapidly three times with phosphate-buffered saline (PBS) and finally lysed and digested with 500 µL of 1 *M* sodium hydroxide. Aliquots of the solubilized cells were counted by a liquid scintillation counter (Packard Instruments Co., USA). The remainder was taken for protein determination by the method of Bradford *(20)*.

Superoxide Anion Production Assay

Superoxide anion production in macrophages was measured by the method described by Hsiao *(18)*. After incubating 5×10^5 cells/well ($n = 8$) in a 96-well microplate with Cr and insulin for 4 h, 50 µL cytochrome-*c* (4 mg/mL; Sigma Chemical Co.) and 50 µL phorbol 12-myristate 13-acetate (PMA) (5 µg/mL; Sigma Chemical Co.) were added in the DMEM and incubated for 30 min at 37°C. The reactions were terminated by placing them on ice. The mixtures were centrifugated at 3000*g* for 3 min at 4°C, and the suspensory fractions were taken for measurement of reduced

cytochrome-*c* production by using a microplate spectrophotometer at 550 nm (Bio-Tek 310, USA). Absorbance values were converted to nanomoles of cytochrome *c* reduced/h using the extinction coefficient 21.1×10^4 *M* cm.

Glucose-6-Phosphate Dehydrogenase Assay

Macrophages were plated at a density of 2×10^7 cell per 75T flask in 20 mL of culture medium ($n = 3$). After incubation for 4 h at 37°C in 5% CO_2, adherent macrophages were washed vigorously with PBS and 1 mL of the extraction medium containing 50 mmol Tris-HCl/L, 1 mmol EDTA/L, and 0.05% (v/v) Triton X-100, pH 8.0, was added. Glucose-6-phosphate dehydrogenase (G-6-PDH; E.C. 1.1.1.49) was assayed by using a G-6-PDH assay kit (Sigma Chemical Co.) according to the modified method of Costa Rosa et al. *(21)*. The amount of enzyme was assayed by following the rate change in absorbance at 340 nm within 5 min at 30°C (Hitachi-2000, USA). A reduction of 1 nmol of $NADP^+$ in 1 s was determined as 1 unit of G-6-PDH.

Phagocytosis

Phagocytosis was studied by coincubation of macrophages with live *Escherichia coli* (ATCC 23815) *(22)*. *E. coli* was grown in 5 mL trypic soy broth (Difco Labs, Detroit, MI, USA) at 37°C for 18 h. After centrifugation at 1000*g* for 25 min, the bacterial pellet was washed twice and resuspended in PBS. The bacterial suspension was sonicated for 10 s to break up clumps of bacteria; the optical density was measured as a basis to adjust the concentration to 2×10^8 bacteria/mL. Immediately prior to assays, bacteria were treated (opsonized) with 10% pig serum for 10 min at 37°C to facilitate phagocytosis. The opsonized bacterial suspension was sonicated again.

Assay was performed ($n = 6$ wells/treatment) by using a 96-well microplate. After incubating 1×10^5 cells/well with Cr and insulin for 4 h, all wells received *E. coli* of 1×10^7 bacteria/well suspended in DMEM. Plates were incubated at 37°C in a shaking water bath for 60 min followed by the addition of 100 µL of DMEM : H_2O (1 : 1) solution and 10 µCi [^{3}H] uridine (specific activity 37.0 Ci/mmol, Amersham) in 25 µL to all wells. The [^{3}H] uridine was incorporated into nonphagocytized bacteria and used to calculate phagocytosis. After 30 min incubation, 20 µL of 1 *M* sodium hydroxide was added to cultures for 3 min, and cells then were harvested (Parkard Inst. Co.) onto glass microfiber filters (934-AH, Whatman, USA). The amount of radioactivity was measured by a liquid scintillation counter. The results were calculated as the phagocytic index (PI), by the following equation:

$$\text{PI} = 1 - \left(\frac{(\text{cpm bacteria} + \text{macrophages})}{(\text{cpm bacteria alone} + \text{cpm macrophage alone})} \times \frac{\text{bacteria}}{\text{macrophage ratio}} \right)$$

Statistical Analysis

The variance for each set of data was analyzed by using the GLM procedure of SAS *(23)*. All data were expressed as mean ± S.E. The significance among treatments was analyzed by Duncan's multiple range test ($p < 0.05$).

RESULTS AND DISCUSSION

The dose-dependent effect of insulin on increasing glucose uptake by macrophages is presented in Table 1. The glucose uptake in cells incubated with $5 \times 10^{-8}M$ insulin was 0.62 nmol/min/mg protein, which was higher than that of the non-insulin-treated control with 0.45 nmol/min/mg protein. Therefore, $5 \times 10^{-8}M$ insulin was chosen for further assays.

Chromium-stimulated changes in glucose uptake of cells differentiated in $CrCl_3$ and CrPic are presented in Fig. 1. The highest glucose uptake occurred after cells were incubated with 20 μg/L Cr of medium containing $CrCl_3$ or CrPic. However, the glucose uptake declined with the addition of 100 μg/L Cr by $CrCl_3$ or CrPic. The increased glucose uptake by the addition of $CrCl_3$ was not dependent on the presence of insulin, but the increased glucose uptake by the addition of CrPic behaved in an insulin-dependent manner.

In Table 2, we tested the effect of the same level of picolinic acid and CrPic (60 μg/L picolinic acid) on glucose uptake. The data showed that the addition of picolinic acid had no effect on glucose uptake. This demonstrated that the enhancing effect of CrPic on glucose uptake was accomplished by CrPic. In an experiment on rat skeletal muscle cells, a similar result was also found *(24)*.

In the present study, the Cr level in medium was 0.46 μg/L. The positive effect of inorganic Cr on glucose uptake to a lesser extent was seen in the absence of insulin, suggesting perhaps an insulin-mimic function of $CrCl_3$. Glucose uptake was lower with the addition of 100 μg/L Cr than 20 μg/L from $CrCl_3$ or CrPic, suggesting a possible saturable mechanism at work or a toxic effect of Cr. In this study, addition of Cr 20 μg/L with $CrCl_3$ increased glucose uptake by 20% and 10% in insulin-treated and control groups, respectively. This result was similar to those in the myotube cells of mouse, in which the addition of 1 μg/L Cr with $CrCl_3$ may have enhanced the maximum response of glucose uptake *(19)*. Chromium picolinate increased glucose uptake only in the presence of insulin (Fig. 1). This showed that the effect of CrPic on glucose uptake was insulin-dependent in macrophages. This result was similar to a study involving liposomal membranes, in which CrPic was shown to enhance insulin internalization *(14)*. Increasing insulin internalization may enhance glucose uptake of muscle cells *(24)*, suggesting that CrPic may act as an essential trace element in insulin-dependent glucose uptake. It has been found that the macrophage surface contains glucose transport proteins *(25)*. Insulin may

Table 1
Effect of Insulin on 2-Deoxy-glucose Uptake in Pig Pulmonary Alveolar Macrophages

Insulin (M)	Glucose uptake+ (nmol/min/mg protein)
0	0.448±0.037
5×10^{-12}	0.497±0.027
5×10^{-11}	0.465±0.052
5×10^{-10}	0.503±0.031
5×10^{-9}	0.569±0.028*
5×10^{-8}	0.617±0.028*
5×10^{-7}	0.725±0.023*

+Values are mean ± S.E. (n = 8).
*Significantly different from basal level ($p < 0.05$).

increase the number of glucose transport proteins; thus, this results in increased glucose uptake in the presence of insulin *(26,27)*.

Changes in O_2^- production following the addition of Cr are shown in Fig. 2. An increase of 1.24-fold and 1.40-fold O_2^- production occurred in those cells incubated with 100 µg/L $CrCl_3$ medium with and without insulin, respectively. The addition of 20 µg/L CrPic increased O_2^- production in the presence of insulin. However, when supplementation 100µg/L CrPic to the medium had enhanced O_2^- production regardless the presence of insulin, the O_2^- production increased 1.27-fold and 1.23-fold in 100 µg/L Cr with CrPic in the incubating medium with and without insulin, respectively. Insulin treatment has no effect on O_2^- production.

The addition of $CrCl_3$ or CrPic increased O_2^- production. This result is similar to previous studies that have suggested the dose-dependent effects of CrPic and chromium nicotinate on enhancing O_2^- production in the cultured cell line of macrophages *(28)*. The addition of $CrCl_3$ and CrPic resulted in a significant increase in the production of O_2^-, and the concentration used in this study was 1000 times lower than the toxic level of Cr (3+) in human macrophage tests *(28–30)*.

The activity of G-6-PDH was not affected by Cr treatment and the addition of insulin to the medium tended to raise the activity of G-6-PDH ($p < 0.12$) (Fig. 3). The average activities of G-6-PDH were 47.43 and 36.26 nmol/min/mg protein in the presence and absence of insulin, respectively. Insulin also has increased the activity of G-6-PDH of bovine microvascular endothelial cells, as a result of stimulating the activity of glycolysis and pentose cycle enzymes *(31)*. The supplementation of 600 ng/g CrPic in the diet resulted in enhanced activity of hexose kinase to normal

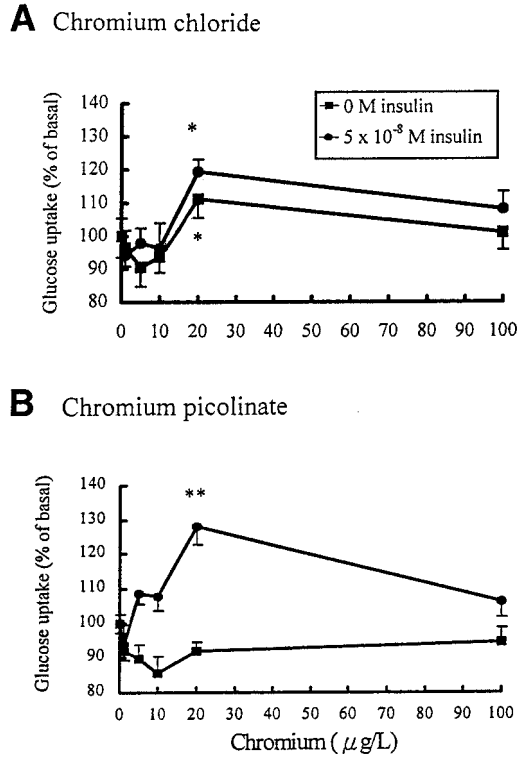

Fig. 1. Effect of chromium chloride **(A)** and chromium picolinate **(B)** on uptake of 2-deoxy-glucose in pig macrophages. Data presented as percentage of basal uptake (basal = 100%) are cells cultured in insulin 0 (■) and $5 \times 10^{-8}M$ (●). Values are mean ± S.E. (n = 8). Significant differences from basal level: $^{**}p < 0.01$ and $^{*}p < 0.05$.

Table 2
Effect of Chromium Picolinate and Picolinic Acid on 2-Deoxy-glucose Uptake in Pig Pulmonary Alveolar Macrophages

Treatment	Insulin (M)	
	0	5×10^{-8}
Control	100.00±2.57[+]	100.00±3.89
Chromium picolinate	103.06±6.11	112.48±3.90[*]
Picolinic acid	99.21±4.40	97.71±4.28

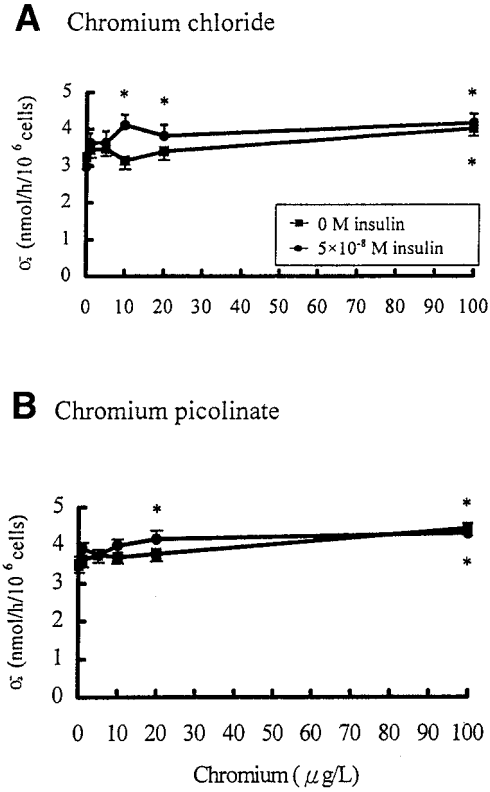

Fig. 2. Effect of chromium chloride **(A)** and chromium picolinate **(B)** on superoxide anion production in pig macrophages. Data presented are cells cultured in insulin 0 (■) and $5 \times 10^{-8}M$ (●). Values are mean ± S.E. (n = 8). Significant difference from basal level: *$p < 0.05$.

in insulin-dependent diabetes mellitus rats *(32)*. The patterns of G-6-PDH and O_2^- production are well correlated with respect to Cr addition (Figs. 2 and 3). The increased activity of G-6-PDH has been implicated in enhancing NADPH production, which may be associated with more O_2^- production *(33)*.

Figure 4 shows the effects of both Cr salts and insulin supplementation on phagocytosis in macrophages. The cumulative effect of the addition of $CrCl_3$ with insulin was highly significant on phagocytosis of macrophages ($p < 0.01$) (Fig. 4). In the presence of insulin, the addition of Cr above 10 μg/L with $CrCl_3$ increased the phagocytosis of macrophages. However, no difference in the addition of $CrCl_3$ without insulin was observed. The addition of 100 μg/L CrPic resulted in higher phagocytosis than that of the 1 and 10 μg/L CrPic groups in the presence of insulin ($p < 0.05$). The phagocytic indexes were 43.29% and 26.99% with the addition of 100 and 1 μg/L CrPic, respectively. However, there were no differences among the addition of CrPic 0 to 10 μg/L in the medium. Supplementation of chelated 0.5 ppm Cr in the diet did not affect the

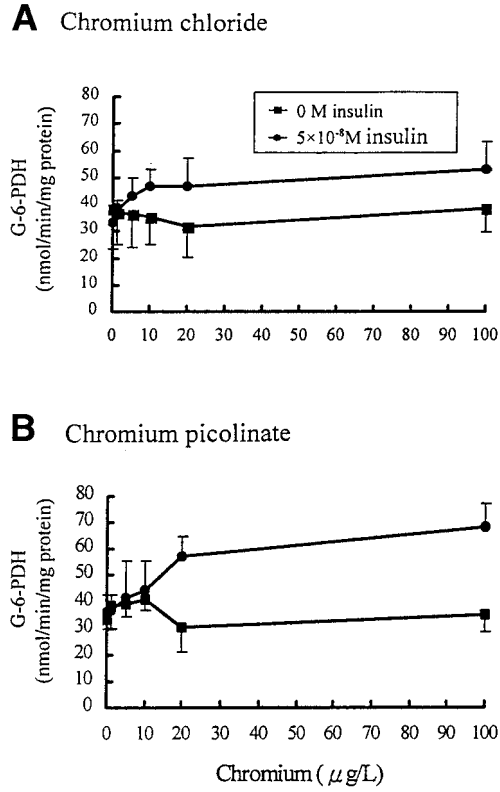

Fig. 3. Effect of chromium chloride **(A)** and chromium picolinate **(B)** on glucose-6-phosphate dehydrogenase activity in pig macrophages. Data presented are cells cultured in insulin 0 (■) and $5 \times 10^{-8}M$ (●). Values are mean ± S.E. (n = 3).

neutrophil phagocytosis of cows *(13)*. However, Cr has been reported to increase the blastogenesis of PBMC *(11,12)*.

Chromium has been known as both a glucose tolerance factor and a low-molecular-weight Cr binding substance, and to exist in trace levels in free form in the body *(34)*. Chromium ions can affect cellular activity by catalyzing the removal of pyrophosphate from nucleoside triphosphate molecules *(35)*. That the addition of $CrCl_3$ resulted in increased glucose uptake and O_2^- production regardless of insulin in this experiment was similar to a previous experiment conducted with muscle cells by Morris et al. *(19)*. The present study confirmed the direct effect of Cr on O_2^- production and phagocytosis in macrophages of weanling pigs.

The compound in the Cr–pyridine carboxylate complexes with best affinity is CrPic. In addition, CrPic has a higher glucose uptake capacity in muscle cells than those of Cr niacinate or $CrCl_3$ *(14,24)*. Further, that CrPic induced glucose uptake in an insulin-dependent manner similar to previous experiment shows that CrPic increased cellular function only in the presence of insulin or in insulin-sensitive tissues *(4)*.

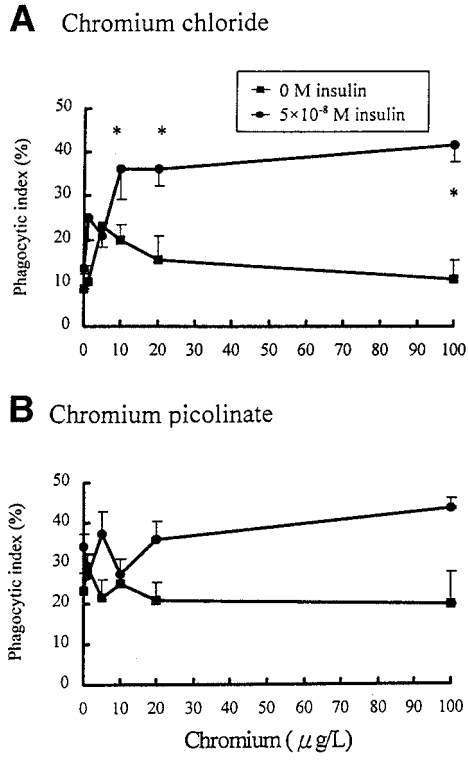

Fig. 4. Effect of chromium chloride **(A)** and chromium picolinate **(B)** on phagocytic index in pig macrophages. Data presented are cells cultured in insulin 0 (■) and $5 \times 10^{-8}M$ (●). Values are mean ± S.E. ($n = 6$). Significant difference from basal level: *$p < 0.05$.

The Cr concentrations of plasma were 3.45 and 5.48 μg/L in the groups supplemented with 0 and 400 ppb Cr from CrPic in weanling pigs, respectively *(7)*. In this study, the measurement of glucose uptake (Fig. 1), superoxide anion production (Fig. 2), glucose-6-phosphate dehydrogenase activity (Fig. 3), and phagocytic index (Fig. 4) showed that there were no differences between $CrCl_3$ and CrPic in vitro. Under in vitro conditions, the concentration for both $CrCl_3$ and CrPic to induce the physiological response of macrophages was 10–20 μg/L which is much higher than the plasma levels of Cr-fed pigs. The viability of human J774A.1 macrophage cells was not affected in a medium containing 30–50 μg/mL CrPic for 24 h *(28)*. This concentration was 1000-fold higher than the effective concentration used in this study. From this point of view, CrPic might to postulated to be a safe agent for macrophages.

Use of this system in vitro to evaluate the effects of Cr on the function of pig macrophages would examine the direct effects of Cr on the immune system. In this regard, the results suggest that the supplementation of 10–20 μg/L Cr with $CrCl_3$ medium enhances the activity of

macrophages directly, whereas the CrPic requires the cooperative action of insulin to enhance glucose uptake and phagocytosis.

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REFERENCES

1. K. Schwarz and W. Mertz, Chromium (III) and the glucose tolerance factor, *Arch Biochem. Biophys.* **85,** 292–295 (1959).
2. W. Mertz, Chromium in human nutrition: a review, *J. Nutr.* **123,** 626–633 (1993).
3. NRC, *Nutrient Requirements of Swine*, 10th rev. ed., National Research Council, Washington, DC (1998).
4. B. W. Morris, T. A. Gray, and S. MacNeil, Glucose-dependent uptake of chromium in human and rat insulin-sensitive tissues. *Clin. Sci.* **84,** 477–482 (1993).
5. E. K. Amoikon, J. M. Fernandez, L. L. Southern, D. L. Thompson, Jr., T. L. Ward, and B. M. Ocott, Effect of chromium tripicolinate on growth, glucose tolerance, insulin sensitivity, plasma metabolites, and growth hormone in pigs. *J. Anim. Sci.* **73,** 1123–1130 (1995).
6. L. D. Bunting, J. M. Fernandez, D. L. Thompson, Jr., and L. L. Southern, Influence of chromium picolinate on glucose usage and metabolic criteria in growing Holstein calves, *J. Anim. Sci.* **72,** 1591–1599 (1994).
7. D. N. Lee, H. T. Yen, T. F. Shen, and B. J. Chen, The effects of chromium picolinate supplementation on growth performance and immunity response of weanling pigs, *J. Chin. Soc. Anim. Sci.* **26,** 373–386 (1997).
8. D. N. Lee, Effects of chromium picolinate supplementation on the growth performance and immune responses of weanling pigs, Ph.D. thesis, National Taiwan University, Taipei, Republic of China (1999).
9. E. van Heugten and J. W. Spears, Immune response and growth of stressed weanling pigs fed diets supplemented with organic or inorganic forms of chromium, *J. Anim. Sci.* **75,** 409–416 (1997).
10. J. L. Burton, B. A. Mallard, and D. N. Mowat, Effects of supplemental chromium on immune responses of periparturient and early lactation dairy cows, *J. Anim. Sci.* **71,** 1532–1539 (1993).
11. J. L. Burton, B. J. Nonnecke, T. H. Elsasser, B. A. Mallard, W. Z. Yang, and D. N. Mowat, Immunomodulatory activity of blood serum from chromium-supplemented periparturient dairy cows, *Vet. Immunol. Immunopathol.* **49,** 29–38 (1995).
12. X. Chang, B. A. Mallard, and D. N. Mowat, Proliferation of peripheral blood lymphocytes of feeder calves in response to chromium, *Nutr. Res.* **14,** 851–864 (1994).
13. X. Chang, B. A. Mallard, and D. N. Mowat, Effects of chromium on health status, blood neutrophil phagocytosis and in vitro lymphocyte blastogenesis of dairy cows, *Vet. Immunol. Immunopathol.* **52,** 37–52 (1996).
14. G. W. Evans and T. D. Bowman, Chromium picolinate increases membrane fluidity and rate of insulin internalization, *J. Inorg. Biochem.* **46,** 243–250 (1992).
15. C. F. Nathan, Secretory products of macrophages, *J. Clin. Invest.* **79,** 319–326 (1987).
16. U. Orlinska and R. C. Newton, Role of glucose in interleukin-1β production by lipopolysaccharide-activated human monocytes, *J. Cell. Physiol.* **157,** 201–208 (1993).
17. D. P. Speert and S. Gordon, Phagocytosis of unopsonized pseudomonas aeruginosa by murine macrophages is a two-step process requiring glucose, *J. Clin. Invest.* **90,** 1085–1092 (1992).

18. S. H. Hsiao, The effect of recombinant porcine interleukin-2 on the immune response of porcine inactivated viral vaccines. M. S. thesis, National Taiwan University, Taipei, Republic of China (1996).
19. B. Morris, T. Gray, and S. MacNeil, Evidence for chromium acting as an essential trace element in insulin-dependent glucose uptake in cultured mouse myotubes, *J. Endocrinol.* **144,** 135–141 (1995).
20. M. M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein, utilizing the principle of protein–dye binding, *Anal. Biochem.* **72,** 248–254 (1976).
21. L. F. B. P. Costa Rosa, Y. Cury, and R. Curi, Effects of insulin, glucocorticoids and thyroid hormones on the activities of key enzymes of glycolysis, glutaminolysis, the pentose–phosphate pathway and the Krebs cycle in rat macrophages, *J. Endocrinol.* **135,** 213–219 (1992).
22. I. A. Rajkovic and R. Williams, Rapid microassays of phagocytosis, bacterial killing, superoxide and hydrogen peroxide production by human neutrophils in vitro, *J. Immunol. Methods* **78,** 35–47 (1985).
23. SAS, *SAS/STAT User's Guide (Release 6.02)*, SAS Institute Inc., Cary, NC (1987).
24. G. W. Evans and D. J. Pouchnik, Composition and biological activity of chromium-pyridine carboxylate complexes, *J. Inorg. Biochem.* **49,** 177–187 (1993).
25. M. Fukuzumi, H. Shinomiyi, Y. Shimizu, K. Ohishi, and S. Utsumi, Endotoxin-induced enhancement of glucose influx into murine peritoneal macrophages via GLUT1, *Infect. Immunol.* **64,** 108–112 (1996).
26. D. Daneman, B. Zinman, M. E. Elliot, P. J. Bilan, and A. Klip, Insulin-stimulated glucose transport in circulating mononuclear cells from nondiabetic and IDDM subjects, *Diabetes* **41,** 227–234 (1992).
27. J. F. Hofert and K. L. Phillips, In vitro insulin-stimulated conversion of (U-^{14}C) glucose to $^{14}CO_2$ by rat thymocytes, *Endocrinology* **102,** 751–756 (1978).
28. D. Bagchi, M. Bagchi, J. Balmoori, X. Ye, and S. J. Stohs, Comparative induction of oxidative stress in cultured J774A.1 macrophage cells by chromium picolinate and chromium nicotinate, *Res. Comm. Mol. Pathol. Pharm.* **97,** 335–346 (1997).
29. D. Bagchi, E. A. Hassoun, M. Bagchi, and S. J. Stohs, Chromium-induced excretion of urinary lipid metabolites, DNA damage, nitric oxide production, and generation of reactive oxygen species in Sprague–Dawleys rats, *Comp. Biochem. Physiol.* **110,** C177–187 (1995).
30. E. A. Hassoun and S. J. Stohs, Chromium-induced production of reactive oxygen species, DNA single-strand breaks, nitric oxide production, and lactate dehydrogenase leakage in J774A.1 cell cultures, *J. Biochem. Toxicol.* **10,** 315–321 (1995).
31. G. Wu, S. Majumdar, J. Zhang, H. Lee, and C. J. Meininger, Insulin stimulates glycolysis and pentose cycle activity in bovine microvascular endothelial cells, *Comp. Biochem. Physiol.* **108,** 179–185 (1994).
32. G. S. Morris, D. L. Hasten, M. Hegsted, and L. K. Guidry, Chromium picolinate supplementation improves cardiac metabolism, but not myosin isoenzyme distribution in the diabetic heart, *J. Nutr. Biochem.* **7,** 617–622 (1996).
33. A. R. Cross and O. T. G. Jones, Enzymic mechanisms of superoxide production, *Biochim. Biophys. Acta* **1057,** 281–298 (1991).
34. E. G. Offenbacher and F. X. Pi-Sunyer, Chromium in human nutrition, *Annu. Rev. Nutr.* **8,** 543–563 (1987).
35. B. Gulanowski, J. Swiatek, and H. Kozlowski, Impact of chromium ions on nucleoside triphosphates and nucleic acids, *J. Inorg. Biochem.* **48,** 289–298 (1992).