Effects of Chromium Picolinate on *In Vitro* Lipogenesis and Lipolysis in Adipose Tissue and Protein Synthesis in Liver Tissue of Pigs

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ABSTRACT : The effects of chromium picolinate supplementation in pig diet were evaluated by measuring the *in vitro* lipogenic and lipolytic activities in adipose tissue and the protein synthetic activity in liver acinar cell in culture. Thirty-two male and thirty-two female pigs were randomly assigned to one of four dietary groups: Control, 100 ppb, 200 ppb, and 400 ppb of Cr in the form of picolinate. The chromium picolinate supplementation ($p < 0.01$) increased the *in vitro* lipolytic activity in adipose tissue of pig, but had no effects on lipogenesis. The chromium picolinate effect was greater in female pigs than in male pigs on lipolytic activity. The results from the studies with the liver acinar cells in culture indicated that chromium picolinate supplementation increased protein synthetic activity ($p < 0.05$). It was observed through this experiment that chromium picolinate functions not only on fat degradation but also on retained protein synthesis.

(**Key Words:** Chromium Picolinate, Lipogenesis, Lipolysis, Acinar Cell Culture, Pig)

INTRODUCTION

Chromium has been recognized as an essential element for mammals since Schwartz and Mertz (1959) first elucidated the significance of this metal in normal glucose utilization, while chromium is toxic like other micronutrients when given in excessive amounts. It was known that chromium was widely distributed throughout the body, and was carried by the fractions of the serum proteins and was rapidly taken up by other tissues (Mertz, 1969; Mertz and Roginski, 1971). But little is known about the chemical form of chromium in the tissues or serum proteins, although "glucose tolerance factor" was found as a natural chromium complex occurring in brewer's yeast and was assumed to activate the action of insulin and membrane transport (Mertz, 1969). Several investigations with both animals and humans provide evidence that the chromium appears to be required for the action of insulin in controlling glucose metabolism. Chromium deficiency in both humans and animals resulted in impaired glucose tolerance, elevated blood glucose levels, hyper-cholesterolemia and development of aortic plaques. The exact mechanism whereby chromium participates in the function of insulin has not been elucidated but the theory ranged from a direct interaction of chromium with insulin to a role of chromium in the production of insulin receptors (Mertz, 1969; Evans et al., 1973; Anderson, 1986). To date, the synergism between chromium and insulin has been studied primarily with regard to the hormone's regulation of carbohydrate and lipid metabolism. However, because insulin also has a key role in muscle metabolism (Felig, 1975), depletion of chromium in the body may impair the development of lean body mass. Glucose-independent effects of chromium on amino acid transport and utilization for protein synthesis also have been shown (Okada et al., 1983; 1984). Most diets for animal are primarily composed of ingredients from plant organ, which are usually low in chromium (Schroeder, 1971). The absorption and utilization of chromium may be dependent on its association with an organic molecule such as picolinate (Mertz, 1969; Evans and Johnson, 1980b). Although several investigations were conducted to evaluate the effects of chromium picolinate supplementation on glucose metabolism, studies on the effects of chromium picolinate on fat and protein metabolism was rare. Therefore, the purpose of this investigation was to assess the effects of chromium on *in vitro* lipogenesis and lipolysis in adipose tissue and protein synthesis in liver tissue of pigs fed diets containing different levels of chromium picolinate.

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MATERIALS AND METHODS

Experimental design

As shown in table 1, experimental diets were formulated to contain four different levels of chromium picolinate (0, 100, 200 and 400 ppb of chromium picolinate) within diets suggested by NRC (1988). A total of 64 cross-bred pigs in which male and female pigs were 32, respectively, with similar body weight were used in this study. Animals were randomly alloted into pens and were fed diets supplemented with 0, 100, 200, or 400 ppb of chromium picolinate. Male and female pigs were randomly assigned to each dietary group (n=64; 4 treatments × 4 replictations × 4 pigs (male 2; female 2)). Upon termination of feeding trial, two pigs selected from each replicate were slaughtered to obtain adipose tissue from backfat and liver tissue. *In vitro* lipogenic activity and lipolytic activity were investigated with adipose tissue. To measure protein synthetic activity, liver acinar cells samples were prepared by pooling four culture dishes of same cell number (about 10^6 cells/35 × 10 mm dish) into one centrifuge tube (50 ml) per same treatment. Therefore, the pooled acinar cells of each treatment were cultured in four observations and four pooled dishes were set up for each observation.

Table 1. Ingredients and chemical composition of the basal diets[1]

	Growing	Finishing
Ingredients:	..... %	
Corn, yellow	77.40	80.80
Soybean meal	19.20	16.80
Fish meal	1.20	–
Limestone	0.75	1.00
TCP	0.65	0.92
Salt	0.32	0.35
L-lysine		0.13
Vit. & Min.premix[2]	0.20	0.20
Antibiotics	0.15	–
Total	100.00	100.00
Composition[3]:		
ME. (kcal/kg)	3,300	3,306
Crude protein (%)	16.00	14.00
Ca (%)	0.60	0.70
P (%)	0.50	0.50
Lysine (%)	0.80	0.70

[1] Formulated to meet the nutrient requirements suggested by NRC (1988).

[2] The vitamin and mineral premix contained the following per kg: vitamin A, 2,000,000 IU; vitamin D_3, 400,000 IU; vitamin E, 250 IU; vitamin K, 200 mg; d-panthothenic acid, 3,000 mg; vitamin B_1, 200 mg; vitamin B_2, 700 mg; vitamin B_6, 200 mg; vitamin B_{12}, 2,200 mg; niacin, 8,000 mg; choline chloride, 30,000 mg; folic acid, 400 mg; BHT, 6,000 mg; Mn, 12,000 mg; Cu, 500 mg; Fe, 4,000 mg; Zn, 15,000 mg; I, 250 mg; Co, 100 mg; Mg, 2,000mg.

[3] Calculated values.

Reagents

Eagle's modified minimum essential medium (MEM), balanced salt solution (BSS), penicillin, amphotericin B, streptomycin, collagenase (Type I), hyaluronidase (Type I), trypsin and antibiotics were obtained from Sigma Chemical Company (St. Louis, MO, USA). Fetal bovine serum and calf serum were purchased from GIBCO BRL (Grany Island, NY, USA). The [^{3}H]-lysine and [^{14}C]-glucose were obtained from Amersham (Buckinghamshire, UK). Other reagents were obtained from Sigma Chemical Company (St. Louis, MO, USA).

Measurement of lipogenic activity

Adipose tissues were sliced with scissors. Tissue slices (10-20 mg) were put into a vial (10 ml) containing 3 ml of Krebs-Ringer bicarbonate buffer with addition of 25 mM HEPES (N-[2-Hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid]), 5.0 mM glucose, 3% bovine serum albumin and 0.5 μCi [^{14}C]-glucose. After incubation of the vials at 37℃ for 2 hrs under a gaseous atmosphere of 5% CO_2 in O_2, tissue slices were taken out from the buffer solution and total lipids were extracted from the slices by following the method of Dole and Meinertz (1960). The extracts were dried by evaporating organic solvents used in the extraction. Radioactivity of [^{14}C]-glucose incorporated into the total lipids was determined in a liquid scintillation counter (LS 100C Beckman).

Measurement of lipolytic activity

Lipolytic activity in the adipose tissue was measured in Krebs-Ringer bicarbonate buffer along with 50% $CaCl_2$ concentration, 4% fatty-acid-poor Fraction V bovine serum albumin and 5.56 mM glucose. Tissue slices were incubated in 3 ml of the KRB buffer solution at 37℃ for 2 hrs under a gaseous atmosphere of 5% CO_2 in O_2. Incubations were terminated by placing the vials containing the tissue slices on ice. The KRB buffer solution was filtered through several layers of cheesecloth to remove the tissue slices and stored at −20℃ until analysis. Non-esterified fatty acids (NEFA) in the medium were extracted and titrated according to the method of Kelly (1965).

Preparation of liver acinar cells

Pig liver was obtained immediately after slaughter, bathed in sterile BSS containing antibiotics (Penicillin[100 U/ml], Amphotericin-B[2.5 μg/ml], and Streptomycin[100 U/ml]. Cell dispension was accomplished by following the method of Choi et al. (1988), as described below. Liver tissues were trimmed to remove other tissues and minced with sterile scissors to less than 5 mm^3 in a solution of 400 U/ml of collagenase (Type I), 400 U/ml hyaluronidase (Type I), 5% (v/v) fetal bovine serum, and 0.15% (v/v) trypsin in 1 × MEM. The solution containing minced tissues was incubated at 37℃ for 3 hrs with a continuous and gentle stirring. Then the cell suspension was filtered through four layers of cheesecloth and the disaggregated cells filtered out were precipitated by centrifugation at 1,000 × g at 4℃ for 5 min. Precipitated cells were washed twice in BSS and resuspended in 1 × MEM. The cells were plated on plastic tissue culture dishes (about 10^6 cells/35 × 15 mm dish). In all studies, four dishes were set up for each observation, and pooled after incubation for subsequent analysis.

Acinar cell culture and measurement of protein synthetic activity

Liver cells were maintained in Eagle's 1 × MEM. Glucose and bovine serum were added to the media to final concentrations of 0.2%(W/V) and 5% (V/V), respectively. Antibiotics (Penicillin 100U, Amphotericin-B 2.5 μg, Streptomycin 100U per 1 ml media) were added to the media. The pH of the media was adjusted to 7.4 by addition of 7.5% sodium bicarbonate. To label the proteins to be synthesized, 0.5 μCi [^{3}H]-lysine was added to the media (1 ml). At the termination of the 18 h incubation, cells were collected, pooled (four dishes for each observation of each treatment), and centrifuged at 1,000 g at 4℃ for 10 minutes. The specific activity of protein synthesis was measured as described previously (Choi et al., 1988). Specific activity of [^{3}H]-lysine incorporated into protein was counted in Insta gel by a liquid scintillation counter (LS 100C, Beckman).

Statistical analysis

All data were analyzed according to a completely randomized design. The differences of means between

Table 2. Effects of chromium picolinate (CrP) on growth performance in growing-finishing pigs

Items	CrP[1] (ppb)				SEM[2]
	0	100	200	400	
			Growing phase (56 d)		
Initial wt (kg)	20.1	19.9	19.8	19.8	
Final wt (kg)	64.8	64.8	64.6	64.6	0.91
Average daily gain (g/d)	779	802	799	800	15.6
Feed intake (kg/d)	2.30	2.34	2.32	2.24	0.038
Feed : gain	2.88	2.92	2.91	2.81	0.057
			Finishing phase (47 d)		
Initial wt (kg)	64.8	64.8	64.6	64.6	0.91
Final wt (kg)	106.8	106.0	103.7	106.1	0.96
Average daily gain (g/d)	893	876	832	884	19.2
Feed intake (kg/d)	3.25[a]	3.12[a]	3.00[b]	3.12[b]	0.049
Feed : gain	3.63	3.56	3.61	3.54	0.060
			Growing-finishing phase (103 d)		
Initial wt (kg)	20.1	19.9	19.8	19.8	0.12
Final wt (kg)	106.8	106.0	103.7	106.1	0.96
Average daily gain (g/d)	842	836	814	838	8.9
Feed intake (kg/d)	2.73[a]	2.70[ab]	2.63[b]	2.64[b]	0.024
Feed : gain	3.24	3.23	3.23	3.16	0.031

[1] Values are means of four replicates consisting of 4 pigs each.
[2] Standard error of mean.
[ab] Means with different superscripts within the same row are significantly different ($p < 0.01$).

treatments were compared by Duncan's multiple range test, using General linear Model (GLM) procedures of SAS package (SAS, 1985).

RESULTS AND DISCUSSION

As shown in table 2, feed intake was about same among the dietary groups, but average daily gain tended to increase. Change of *in vitro* lipogenic and lipolytic activites in adipose tissue of pigs fed diets containing different levels of chromium picolinate are shown in table 3. In male pigs, the amount of glucose incorporated into total lipids in adipose tissues were 100.39, 84.90, 82.20 and 79.59 nmol/mg for the control, 100, 200 and 400 ppb dietary group, respectively. In female pigs, the amount of glucose incorporated into total lipids in adipose tissue of female pigs were 123.79, 87.64, 81.54 and 79.49 nmol/mg for control, 100, 200 and 400 ppb dietary group, respectively. As shown in table 3, there were significant differences among treatments, but the lipogenic activites were decreased with increasing amount of supplemention. Although there was no significant difference, male pigs showed lower lipogenic activity than female pigs.

The results for the lipolytic activities are shown in table 3. The highest lipolytic activity in male pigs was observed with the 400 ppb dietary group and in female pigs with the 200 ppb dietary group ($p < 0.01$). Although there were no significant differences between male and female pigs, these results indicated that lipolytic activity was significantly increased in the group fed diet supplemented with 200 ppb of chromium picolinate both in male and female pigs ($p < 0.01$). As there was a significant interaction of sex and chromium picolinate level ($p < 0.01$), this result represented that chromium picolinate had a great influence on lipolytic activity of female pigs than that of male pigs.

As shown in table 4, protein synthetic activity depended on supplementation of chromium picolinate and gender. Secreted protein was not significantly affected by supplemented chromium picolinate levels in both male and female pigs, and there was no significant difference between male and female pigs. In the results of assay of retained protein, however, according to increase in added level of chromium picolinate, amount of retained protein was increased in male pigs. Consequently, the highest retained protein was observed in 400 ppb as 1,653.4 dpm/mg in male and 200 ppb as 1,618.1 dpm/mg in female pigs ($p < 0.05$). In comparison with male and female pigs, the retained protein synthesis was higher in female pig than that of male ($p < 0.05$).

It is now quite clear in table 1 that chromium picolinate increased lipolytic activity in adipose tissue of pigs. Several investigators have measured physiological changes from chromium treated animals. For example, McCarty (1993) showed that chromium picolinate reduced body fat in rat and Evans (1989) reported that chromium picolinate (200 μg/day) increased lean body mass and body fat loss of men in a weight training program. A recent study demontrated that total cholesterol, LDL (low density lipoprotein)-cholesterol and the related transport protein apolipoprotein B were significantly decreased while apolipoprotein A, the HDL (high density lipoprotein) -cholesterol related protein, was elevated by supplementation of chromium picolinate in diets. In view

Table 3. Lipogenic and lipolytic activities of adipose tissue from pigs fed different level of chromium picolinate

	Dietary groups	Lipogenic activity[1]	Lipolytic activity[2]
Sex			
Male	Control	100.39	6.76^{bc}
	100 ppb	84.90	5.60^{bc}
	200 ppb	82.20	7.07^{bc}
	400 ppb	79.59	7.66^{b}
Female	Control	123.79	5.08^{c}
	100 ppb	87.64	6.59^{bc}
	200 ppb	81.54	10.14^{a}
	400 ppb	79.49	6.50^{bc}
Mean		89.93	6.87
PSE[3]		4.62	0.34
Probability (P)		0.4054	0.001
Male		86.77	6.77
Female		93.09	6.97
Chromium level			
Control		112.05	5.93^{b}
100 ppb		86.27	6.10^{b}
200 ppb		81.87	8.61^{a}
400 ppb		79.54	6.86^{b}
Probability (P)			
Sex		0.5128	0.7005
Chromium level		0.1079	0.0023
Sex × Chromium level		0.7868	0.0045

[1] nmol glucose incorporated into total lipids/mg.
[2] μ eq non-esterified fatty acid (NEFA) release/mg.
[3] PSE = pooled standard error of the mean.
^{a,b,c} Means values with different superscript with same column are significantly different ($p < 0.05$).

Table 4. Comparison of protein synthetic activity by liver acinar cell culture

	Dietary groups	Secreted protein[1] (dpm/mg)	Retained protein[2] (dpm/mg)
Sex			
Male	Control	2,465.9	1,002.5^{b}
	100 ppb	2,279.8	1,034.3^{b}
	200 ppb	2,242.6	1,421.5^{ab}
	400 ppb	2,275.9	1,653.4^{a}
Female	Control	2,356.5	1,404.0^{ab}
	100 ppb	2,036.0	1,468.8^{ab}
	200 ppb	2,115.2	1,618.1^{a}
	400 ppb	2,697.5	1,584.8^{a}
Mean		2,608.70	1,398.14
PSE[3]		82.07	63.77
Probability (P)		0.6413	0.0254
Male		2,316.1	1,277.9^{b}
Female		2,301.3	1,518.9^{a}
Chromium level			
Control		2,411.2	1,203.3^{c}
100 ppb		2,157.9	1,251.6^{bc}
200 ppb		2,178.9	1,519.8^{ab}
400 ppb		2,486.7	1,619.1^{a}
Probability (P)			
Sex		0.9314	0.0339
Chromium level		0.4310	0.0295
Sex × Chromium level		0.5221	0.3312

[1] The amount of secreted protein was determined by the incorporation of [^{3}H]-lysine (0.5 μCi/ml) into TCA-insoluble material.

[2] The amount of retained protein was determined by the incorporation of [^{3}H]-lysine (0.5 μCi/ml) into acini.

[3] PSE = Pooled standard error of the mean.

[a,b,c] Means values with different superscripts with same column are significantly different ($p < 0.05$).

of the results of our experiment, as Mersmann and MacNeil (1985) suggested that an increase in NEFA (non-esterified fatty acid) would imply that the process of fat degradation or lipolysis in adipose tissue was increased, chromium picolinate probably reduced body fat in pigs by enhancing lipolysis, rather than by inhibiting adipose lipogenesis.

The effects of chromium picolinate on retained protein and secreted protein in liver acinar cells in culture are summarized in table 2. As Britton et al. (1968) reported that chromium supplementation increased nitrogen retention in lambs, chromium picolinate supplementation in pig diet increased protein synthesis. Especially, increased protein synthesis was caused by not secreted protein but retained protein. This result might suggest that these increases in retained protein are in part due to induction of gene expression, but also could be due to decreased rates of RNA degradation as shown by large increases in the cytoplasmic ribonuclease inhibitor. In conclusion, chromium picolinate had a direct effect on increase of lipolytic activity in adipose tissue and retained protein synthetic activity in *in vitro* liver acinar cell culture. We concluded that although these results were obtained by *in vitro* assay, chromium picolinate might be supplemented in pig diet with 200 ppb in order to decrease fat content and increase protein content in carcass composition.

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